IN THE UNITED STATES DISTRICT COURT

FOR THE DISTRICT OF DELAWARE

BRASSICA PROTECTION PRODUCTS, LLC and JOHNS HOPKINS UNIVERSITY Plaintiffs, v. THE SPROUTMAN INC. and MURRAY TIZER Defendants.	)	Civil Action No.

COMPLAINT FOR PATENT INFRINGEMENT

Plaintiffs, Brassica Protection Products, LLC and Johns Hopkins University, by and through their undersigned counsel, file this Complaint against Defendants, The Sproutman Inc. and Murray Tizer, averring as follows:

NATURE OF THE ACTION

1. This is an action for patent infringement arising under the Patent Laws of the United States, in particular 35 U.S.C. §§ 271, 283, 284 and 285.

THE PARTIES

2. Plaintiff, Brassica Protection Products, LLC ("Brassica"), is a Limited Liability Company organized and existing under the laws of Delaware, having a place of business at 600 East Lombard St., Suite 522, Baltimore, Maryland, 21202.

3. Plaintiff, Johns Hopkins University ("Johns Hopkins"), is a corporation organized and existing under the laws of Maryland, having a principal place of business at 33rd St. and Charles St., Baltimore, Maryland, 21218.

EXHIBIT 3

4. Upon information and belief, defendant, The Sproutman Inc. ("Sproutman"), is a corporation organized and existing under the laws of the Commonwealth of Pennsylvania, having a place of business at 1415 Chestnut Ridge Rd., Upper Black Eddy, Pennsylvania, 18972.

5. Upon information and belief, defendant Murray Tizer is the President and Chief Executive Officer of Sproutman, which has a place of business at 1415 Chestnut Ridge Rd., Upper Black Eddy, Pennsylvania, 18972.

JURISDICTION AND VENUE

6. This court has jurisdiction over the subject matter of this action under 28 U.S.C. § 1338(a).

7. Venue is proper in this district court pursuant to 28 U.S.C. §§ 1391(b) and (c), and 1400(b).

8. Upon information and belief, defendants are doing business in the State of Delaware and have committed and/or induced others to commit acts of infringement in the State of Delaware. Upon information and belief, this Court has personal jurisdiction over Sproutman and Murray Tizer

THE PATENT

9. On March 10, 1998, United States patent 5,725,895 ("the '895 patent") entitled "Method Of Preparing A Food Product From Cruciferous Seeds" was duly and legally issued to Johns Hopkins as assignee of the inventors Jed W. Fahey and Paul Talalay, M.D. A copy of the '895 patent is attached as Exhibit A.

10. Plaintiff Brassica is the exclusive licensee under the '895 patent, and under the terms of its license, Brassica has the right to sue to enjoin any and all infringement of the

'895 patent and to recover damages caused by such infringement. Further, the U.S. Government has a paid-up license under the '895 patent and the right in limited circumstances to require the patent owner to license others on reasonable terms as provided for by the terms of grant PO1 CA 44530, entitled "Novel Strategies for Chemoprotection Against Cancer," awarded by the National Cancer Institute, Department of Health and Human Services.

11. Dr. Talalay is the John Jacob Abel Distinguished Service Professor at the Johns Hopkins University School of Medicine, a member of the National Academy of Sciences of the U.S.A. and the American Philosophical Society. He is also a Fellow of the American Academy of Arts and Sciences and was appointed a life-time Professor of the American Cancer Society. Mr. Fahey is a plant physiologist in the Department of Pharmacology and Molecular Sciences at The Johns Hopkins University School of Medicine. Before joining the Johns Hopkins faculty, he spent 15 years in the biotechnology industry and held senior management positions in agricultural biotechnology research and process development.

12. The invention of the '895 patent is based, in part, upon Dr. Talalay's life-long study of cancer and pursuit of edible plants that have a chemoprotective effect against cancer. Chemoprotection is a means for preventing cancer by increasing the body's own cancer-fighting defense mechanisms by administration of anti-cancer agents delivered, ideally, in the diet. Chemoprotection takes advantage of the ability of cells of the human body to produce a family of detoxification enzymes that neutralize highly reactive and dangerous forms of cancer-causing chemicals before those chemicals can damage DNA and initiate the process that can lead to malignancy. By inducing the production of these detoxification enzymes in the body, protection against cancer can be achieved.

13. Dr. Talalay and colleagues have received worldwide acclaim for their discovery that certain varieties of vegetables, such as broccoli, contain natural chemical agents that are potent inducers of cellular detoxification enzymes that protect against cancer-causing chemicals.

14. Based upon these discoveries, Dr. Talalay founded the Brassica Chemoprotection Laboratory at Johns Hopkins University with support from, inter alia, the National Cancer Institute. The mission of this laboratory was to examine the chemoprotective properties of plants and to ensure that consumption of the most promising vegetables by humans is both safe and effective.

15. Dr. Talalay's examination of the chemoprotective properties of plants was initially focused on mature, market stage vegetables, such as broccoli. However, Dr. Talalay and Mr. Fahey discovered that the concentrations of cancer-fighting enzymes in cruciferous plants are considerably higher during the sprout stage than at later stages of development. They discovered that sprouts of cruciferous seeds, such as broccoli sprouts, can be selected to produce food products that are rich in glucosinolates. Glucosinolates are precursors of chemicals that induce production of cancer-fighting enzymes in the body.

16. Dr. Talalay and Mr. Fahey were awarded the '895 patent for their important discovery. The '895 patent discloses and claims a novel method of preparing a food product which involves germinating certain cruciferous seeds, such as broccoli seeds, and harvesting the sprouts prior to the 2-leaf stage to produce a food product that is rich in glucosinolates.

17. The invention disclosed in the '895 patent has received widespread acclaim in the scientific literature, the popular press and the broadcast media. Dr. Talalay's and

Mr. Fahey's discovery was published in the prestigious scientific journal, *Proceedings of the National Academy of Science of the U.S.A.* on September 16, 1997, and has been the subject of scientific commentary as well as numerous newspaper, magazine, radio and television reports.

18. The importance of the '895 invention also has been recognized by the sprout-growing industry and the public. Prior to the publication of that work in the *Proceedings of the National Academy of Science*, broccoli sprouts were not being grown commercially, nor had they been recognized as a rich source of the cancer chemoprotective compounds. However, within days of that publication in September 1997, broccoli sprouts began to appear in the supermarkets and health food stores.

19. Plaintiff, Brassica, was founded, inter alia, to make Dr. Talalay's and Mr. Fahey's important discovery available to the public. Brassica markets broccoli sprouts rich in glucosinolates that are grown under exacting, hygienic standards. A portion of the proceeds from the product are donated to The Brassica Foundation for Chemoprotection Research Inc. of Baltimore Maryland. The purpose of this foundation is to support research on chemoprotection against cancer by scientists.

COUNT 1

20. The allegations contained in Paragraphs 1 through 19 are incorporated herein by reference as though fully set forth.

21. Upon information and belief, defendant Sproutman has been and now is infringing the '895 patent under 35 U.S.C. § 271(a) and/or (b) by making, using, offering to sell and/or selling and/or actively inducing others to make, use, offer to sell and/or sell the patented invention of the '895 patent. Upon information and belief, Sproutman has known about the '895

patent since at least June 4, 1998. Upon information and belief, Sproutman's infringing activities have been willful and will continue unless enjoined by this Court.

22. Upon information and belief, defendant Sproutman has for a time past and still is infringing and/or inducing infringement of the '895 patent by selling broccoli sprouts utilizing the patented inventions to customers in Delaware through intermediaries and will continue to do so unless enjoined by this Court. Upon information and belief, Sproutman's broccoli sprouts have been in the past and are being sold at, inter alia, Harvest Market, 1252 Old Lancaster Pike, Hockessin, Delaware and the Newark Co-op Natural Foods, 280 E. Main Street, Newark, Delaware.

23. Upon information and belief, defendant Sproutman has profited by its infringing activities; Brassica and Johns Hopkins have been damaged by those infringing activities and will be irreparably injured unless those infringing activities are enjoined by this Court. Brassica and Johns Hopkins do not have an adequate remedy at law.

COUNT 2

24. The allegations contained in Paragraphs 1 through 23 are incorporated herein by reference as though fully set forth.

25. Upon information and belief, defendant Murray Tizer has been and now is infringing the '895 patent under 35 U.S.C. § 271(b) by actively inducing others to make, use, offer to sell and/or sell the patented invention of the '895 patent. Upon information and belief, Murray Tizer has known about the '895 patent since at least June 4, 1998. Upon information and belief, Murray Tizer's infringing activities have been willful and will continue unless enjoined by this Court.

26. Upon information and belief, defendant Murray Tizer has for a time past and still is infringing and/or inducing infringement of the '895 patent by selling broccoli sprouts utilizing the patented inventions to customers in Delaware through intermediaries and will continue to do so unless enjoined by this Court. Upon information and belief, Murray Tizer's broccoli sprouts have been in the past and are being sold at, inter alia, Harvest Market, 1252 Old Lancaster Pike, Hockessin, Delaware and the Newark Co-op Natural Foods, 280 E. Main Street, Newark, Delaware.

27. Upon information and belief, defendant Murray Tizer has profited by his infringing activities; Brassica and Johns Hopkins have been damaged by those infringing activities and will be irreparably injured unless those infringing activities are enjoined by this Court. Brassica and Johns Hopkins do not have an adequate remedy at law.

WHEREFORE, Brassica and Johns Hopkins respectfully request that this Court enter the following relief:

A. A judgment that Sproutman and Murray Tizer have infringed the '895 patent and that their infringement has been willful;

B. A preliminary and permanent injunction enjoining Sproutman, its officers, agents, servants, employees, and attorneys, including defendant Murray Tizer, and those persons in active concert or participation with them who receive actual notice of the order by personal service or otherwise, from any further infringement of the '895 patent;

C. A judgment in favor of Brassica and Johns Hopkins for its damages caused by defendants' infringement and that those damages be trebled and awarded to Brassica and Johns Hopkins with prejudgment interest;

D. A judgment in favor of Brassica and Johns Hopkins for its attorneys fees, costs, and expenses in this action; and

E. A judgment in favor of Brassica and Johns Hopkins for such further necessary proper relief as this Court may deem just.

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Hortus Third

A Concise Dictionary of Plants Cultivated in the United States and Canada

Initially Compiled by
LIBERTY HYDE BAILEY
and ETHEL ZOE BAILEY

Revised and Expanded by
THE STAFF OF THE
LIBERTY HYDE BAILEY HORTORIUM

A Unit of the
New York State College of Agriculture and Life Sciences
a Statutory College of the
State University at Cornell University

Volume One
A-K

Willd.; *Statice pseudarmeria* J. Murr.). Resembling *A. maritima*, but with lvs. broader, lanceolate, to 10 in. long, ¾ in. wide, 5–7-nerved, margins narrowly scarious; involucral sheath to 4 in. long; fls. white to dark rose-pink. Portugal. The commonly cult. broad-lvd. thrift.

rigida: *A. plantaginea.*

Rouyana Daveau. To 1 ft., forming mats; lvs. filiform or linear, channelled, 1-nerved, to 4 in. long; interfloral bracts pubescent at least on basal half, usually as long as or longer than calyx tube, calyx spur more than half as long as calyx tube, calyx hairy on and between ribs, fls. rose-pink. Portugal.

rumelica Boiss. Lvs. stiff, 3–5-nerved, of 2 kinds, outer lvs. broadly linear, shortly acuminate, inner lvs. narrowly linear; fls. purplish-pink. Balkan Pen.

ruscinonensis: *A. maritima.*

setacea: see *A. juncea*. Var. **alba:** a listed name, probably referring to *A. juncea* cv.

sibirica: *A. maritima.*

splendens: *A. juniperifolia.*

stenophylla: *A. plantaginea.*

Sundermanii: a listed name of no botanical standing, used for a form of *A. maritima.*

vulgaris: *A. maritima.* Vars. **nana** and **splendens:** listed names of no botanical standing, used for forms of *A. maritima.*

Walterana: a listed name of no botanical standing.

Welwitschii Boiss. [*A. Welwitschii* var. *stenophylla* Daveau]. Tufted, subshrubby, to 1 ft., glabrous, somewhat glaucous; lvs. linear, to 4 in. long, channelled or flat, obscurely 1-nerved; fls. pink, calyx with basal spur. Portugal.

Willkommiana: *A. maritima.*

→ **ARMORACIA** P. Gaertn., B. Mey. & Scherb. *Cruciferae.* About 3 spp. of glabrous per. herbs, with deep roots or rhizomes, native to Eur. and Asia; lvs. simple to pinnatifid, basal lvs. large and often docklike, variously dissected; fls. white, small, sepals and petals 4; fr. a globose to ellipsoidal silicle.

One species is widely grown as a condiment plant, the fleshy roots being grated for use as a pungent relish or appetizer with meats. Does not mature viable seeds; propagated by root cuttings planted in spring, and best treated as an annual crop, the roots harvested in late autumn of the same year.

rusticana P. Gaertn., B. Mey. & Scherb. [*Cochlearia Armoracia* L.; *Nasturtium Armoracia* (L.) Fries; *Radicula Armoracia* (L.) B. L. Robinson; *Rorippa Armoracia* (L.) A. S. Hitchc.]. **HORSERADISH, RED COLE.** Deep-rooted, strong per.; lower lf. blades to 15 in. long and 9 in. across, crenate-dentate or jagged, sometimes dissected into linear segms., upper st. lvs. lanceolate to oblong; fls. in terminal panicle. Spring. Se. Eur., naturalized in N. Amer.

ARNEBIA Forssk. *Boraginaceae.* Not cult. A. Echioides: *Echioides longiflorum.*

ARNICA L. *Compositae* (Senecio Tribe). About 30 spp. of rhizomatous, pubescent and also usually glandular per. herbs, native to Eur., Asia, and N. Amer., sts. simple or branched above; lvs. opp.; fl. heads radiate or discoid, 1 to several, rather large, long-peduncled, involucral bracts in 2 rows, herbaceous; disc and ray fls. yellow; achenes slender, cylindrical, pappus of minutely barbed or almost plumose bristles.

Arnicas are grown in the rock garden or border or are colonized in woodsy places. Tincture of arnica, derived from *A. montana*, has medicinal uses. Propagated by seeds or division.

alpina (L.) Olin. To 4–15 in.; st. lvs. 1–4 pairs, lanceolate or oblanceolate, to 4–5 in. long, nearly entire, sessile or the lowermost petioled; heads usually solitary, to 2 in. across, involucre woolly; anthers yellow; pappus white. Circumboreal.

amplexicaulis Nutt. To 2 ft., viscid; st. lvs. 4–10 pairs, ovate to broadly elliptic-lanceolate, to 5 in. long, serrate-dentate, sessile; heads 3–9, 1⅛ in. across; anthers yellow; pappus tan. W. U.S. to Alaska.

betonicifolia: *A. latifolia.*

Chamissonis Less. To 2 ft.; st. lvs. 5–8 pairs, lanceolate-oblong to oblanceolate, to 6 in. long, denticulate or dentate, all sessile and clasping, or the lower ones short-petioled; heads 3–9, 2 in. across; anthers yellow; pappus tan. Nw. U.S. to s. Alaska. Subsp. **foliosa** (Nutt.) Maguire [*A. foliosa* Nutt.]. Lvs. usually entire, the lower ones distinctly petioled. Wyo. and Colo., w. to Calif. and Canada.

Clusii: *Doronicum Clusii.*

cordifolia Hook. To 18 in.; st. lvs. 2–3 pairs, the lower broad-lanceolate to ovate, cordate, long-petioled, mostly coarsely dentate, blades 2–3 in. long, the upper reduced, ovate or lanceolate, sessile; heads solitary, or sometimes 3, 2–3 in. across; anthers yellow; achenes pubescent their whole length, pappus white. S. Dak. to New Mex., w. to Calif., n. to Yukon, also n. Mich. The most desirable sp.

foliosa: *A. Chamissonis* subsp.

fulgens Pursh [*A. pedunculata* Rydb.]. Rhizomes short, rooting, with tufts of tan hairs in axils of old lf. bases, sts. 1–2 ft.; st. lvs. 4 or 5 pairs, oblanceolate, the lower crowded near base, petioled, to 6 in. long, nearly entire, the upper much-reduced, sessile; heads usually solitary, 2–3 in. across; anthers yellow; pappus whitish or light tan. B.C. to Sask., s. to n. Calif. and Colo.

latifolia Bong. [*A. betonicifolia* Greene]. To 2 ft.; st. lvs. 2–4 pairs, ovate to elliptic-lanceolate, 1–3 in. long, dentate, all sessile or the lowermost petioled; heads mostly 1–3; anthers yellow; achenes glabrous or hirsute only in upper half, pappus white. Wyo. and Colo., w. to Calif., n. to s. Alaska.

Lessingii Greene. To 10 in., without glands; lvs. 4–5 pairs in a rosette toward base of st., lanceolate or oblanceolate, 2–4 in. long, entire or denticulate, sessile or short-petioled; heads solitary, nodding, 2 in. across; anthers purple; pappus tan. Alaska, Kamchatka.

longifolia D. C. Eat. Sts. in clumps, to 2 ft.; st. lvs. 5–7 pairs, lanceolate to lanceolate-elliptic, to 4 in. long, entire or denticulate, viscid, sessile; heads 7–30, to 2 in. across; anthers yellow; pappus straw-colored or tan. Mts., Calif. and Colo., n. to Wash. and Mont.

mollis Hook. To 2 ft.; st. lvs. 2–4 pairs, ovate, lanceolate, obovate, or oblanceolate, to 6 in. long, denticulate, sessile or the lower ones petioled; heads mostly 1–3, to about 3 in. across; anthers yellow; pappus tan. Colo. to Calif., n. into Canada, also N.Y. and New Eng. to Gaspé Pen.

montana L. To 2 ft.; st. lvs. about 3 pairs, broadly lanceolate, ovate, or obovate, entire or obscurely denticulate, sessile, upper pair much reduced, the others in a basal rosette, to 5 in. long; heads 1–3 or more, to 3 in. across; anthers yellow; pappus tan. Cent. Eur., s. Scandinavia.

pedunculata: *A. fulgens.*

sachalinensis (Regel) A. Gray. Nearly glabrous, 12–30 in.; st. lvs. 12–20 pairs, lanceolate or oblanceolate, to 6 in. long, serrate, sessile and united; heads 5–15, to 2¼ in. across; anthers purple; pappus tan. Sakhalin Is.

saluensis: a listed name; perhaps for *A. sachalinensis.*

unalaschcensis Less. To 3–12 in.; st. lvs. 3–5 pairs, mostly broadly lanceolate or oblanceolate, to 4 in. long, serrulate, the upper sessile, the lower petioled; heads solitary, about 1¼ in. across; corolla tube glabrous, anthers purple; pappus tan. Japan and islands of Bering Sea. Var. **Tschonoskyi** (Iljin) Kitam. & Hara. Corolla tube pubescent. Japan.

ARONIA Medic. **CHOKEBERRY.** *Rosaceae.* A few spp. of low, deciduous shrubs of N. Amer.; lvs. alt., simple, short-petioled, finely serrate; fls. small, pink or white, in terminal cymes, calyx tube urceolate, sepals 5, petals 5, concave, spreading, styles 3–5, united at base; fr. a small berrylike pome. Often considered a subgenus of *Pyrus.*

Propagated by seeds sown when ripe or stratified, and by suckers, layers, and cuttings of green wood under glass. Useful for colonizing in low places; showy in bloom and the fruit attractive in autumn.

arbutifolia (L.) Pers. [*Pyrus arbutifolia* (L.) L.f.]. **CHOKEBERRY, RED C.** Shrub, tending to form colonies, to 12 ft. or more, young growth tomentose; lvs. broadly oblanceolate to wider, pointed, green and glabrous above, tomentose and pale beneath, crenate-serrate, 1–3¼ in. long; fls. 2–25, about ⅝ in. across, calyx tube tomentose, sepals with stipitate glands; fr. red, ⅜ in. in diam. Nov. Sc. to Ont., Mich., s. to Tex., Fla. Zone 6.

atropurpurea: *A. prunifolia.*

floribunda: *A. prunifolia.*

melanocarpa (Michx.) Elliott [*A. nigra* (Sarg.) Koehne; *Pyrus melanocarpa* (Michx.) Willd.]. Like *A. arbutifolia*, but young twigs, lower lf. surfaces, and calyx tube glabrous, sepals essentially glabrous; fr. black, ⅜ in. in diam. Nfld. to Minn., S.C., Tenn.

nigra: *A. melanocarpa.*

prunifolia (Marsh.) Rehd. [*A. floribunda* (Lindl.) Spach; *Pyrus atropurpurea* (Britt.) L. H. Bailey]. Like *A. arbutifolia*, but sepals glandless or nearly so; fr. purple or purple-black, ⅜ in. in diam. Nfld. to Ont., s. to Va. and Ind. Zone 5.

cucullata (L.) R. Br. Pseudobulbs cylindrical, with a bulbous thickening at base. lvs. filiform, cylindrical, fleshy, to 1 ft. long; fls. 1–2 on peduncles to 6 in. long, sepals and petals white to greenish-white, aging to yellowish, spreading and pendulous, linear, acuminate, to 3 in. long, lip to 3 in. long, white, short-clawed, semicircular, with finely toothed margins, apically prolonged into a slender, acuminate lobe. Late spring–early winter. Trop. Amer.

Digbyana Lindl. [*Laelia Digbyana* (Lindl.) Benth.; *Rhyncholaelia Digbyana* (Lindl.) Schlechter]. Pseudobulbs elongated, jointed, club-shaped; lvs. leathery, elliptic, glaucous-green, to 8 in. long; infl. 1-fld.; fl. showy, fragrant, 4–6 in. across, sepals and petals elliptic, pale green-yellow, lip large, 3 in. long, obscurely 3-lobed, nearly orbicular, involute at base and enveloping column, cream-white with greenish suffusion, upper margins deeply lacerate-fringed, disc with several fleshy ridges. Spring–summer. Cent. Amer. Frequently used as a parent in crosses with spp. and cvs. of *Cattleya*, *Laelia*, and *Sophronitis*, the resultant hybrids possessing large, fringed lips.

fragrans Barb.-Rodr. To 20 in.; lvs. fleshy, cylindrical; raceme much shorter than lf., few-fld., flexuous; fls. fragrant, yellowish-white, with a few purple spots, to 2 in. long, sepals and petals filiform, lip recurved, somewhat shorter than sepals. Autumn. Brazil.

glauca Lindl. [*Laelia glauca* (Lindl.) Benth; *Rhyncholaelia glauca* (Lindl.) Schlechter]. Pseudobulbs club-shaped, to 4 in. long; lvs. leathery, oblong-elliptic, glaucous, to 5 in. long; fls. solitary, nodding, fragrant, on peduncle 4 in. long, sepals linear-elliptic, to 2¼ in. long, olive-green to white or lavender, petals similar to sepals in shape and length, olive-green to whitish, lip to 2 in. long, white or yellowish with rose-pink spot over several reddish stripes in throat, 3-lobed, midlobe squarish-oblong, apiculate at apex. Winter–early spring. Cent. Amer.

nodosa (L.) Lindl. LADY-OF-THE-NIGHT. Pseudobulbs 1–4 ¼ in. long; lvs. to 9 in. long; fls. solitary, short-peduncled, sepals and petals linear, to 3 in. long, greenish-yellow or white, lip white, not toothed. Winter. W. Indies, Cent. Amer., Colombia, Venezuela, Surinam.

Perrinii Lindl. To 10 in., with ascending rhizome; lvs. fleshy, cylindrical; peduncle shorter than lf., 1-fld.; fls. to 2 in. long, sepals and petals greenish-yellow, spreading, filiform, lip white, with yellow-green throat, ovate, short-clawed, acute at apex. Brazil, Paraguay.

BRASSIA R. Br. *Orchidaceae.* About 50 spp. of epiphytes, native to trop. Amer.; pseudobulbs 1–3-lvd.; fls. in lateral racemes, sepals and petals narrow, long-pointed, often tail-like, lip entire, shorter than sepals. For structure of fl. see *Orchidaceae*.

Intermediate greenhouse; for culture see *Orchids*.

Allenii L. O. Williams ex C. Schweinf. Pseudobulbs not developed; lvs. many, forming a broad fan, to 1 ft. long; raceme several-fld., from lf. axils, shorter than lvs.; fls. fragrant, sepals and petals similar, spreading, reddish-tan to olive-ochre, 1¼ in. long, linear-lanceolate, lip nearly orbicular, yellow, with band of reddish-tan and a white disc, cuspidate at apex. Autumn. Panama.

brachiata: *B. verrucosa*.

caudata (L.) Lindl. Pseudobulbs to 6 in. long; lvs. oblong or oblong-elliptic, to 11 in. long, acute; infl. to 1¼ ft. long, 6–15-fld.; sepals and petals greenish-yellow, with brown spots on basal half, lateral sepals to 6 in. long, petals to 1 in. long, lip light yellow with brown spots, with 2 small teeth at apex of callus. Winter–late summer. Trop. Amer., from Fla. to S. Amer.

chlorops Endres & Rchb.f. To 1 ft.; lvs. lanceolate; infl. to 10 in. long, shorter than lvs., loosely few-fld.; fls. small, ¾ in. long, greenish, sepals and petals lanceolate, acuminate, lip linear-lanceolate, with 2 pubescent keels at base. Costa Rica, Panama, Colombia.

Gireoudiana Rchb.f. & Warsz. Pseudobulbs to 5 in. long, 2-lvd.; lvs. oblong or elliptic-oblong, to over 17 in. long; infl. over 2 ft. long, usually 7–10-fld.; sepals linear-lanceolate, to 6 in. long, tapering, cream-colored or greenish-yellow, spotted with brown on basal ⅓, petals to 2¼ in. long, yellow, spotted with brown on lower half, lip light yellow, spotted with brown. Early winter–late spring. Costa Rica, Panama.

guttata: *B. maculata*.

Keiliana Rchb.f. ex Lindl. Pseudobulbs much-compressed, to 2 in. long, 1-lvd.; lvs. narrowly ovate, strap-shaped, to 10 in. long; infl. as long as lvs. or longer, few- or many-fld., fl. bracts as long as pedicelled ovary or longer; fls. yellow, spotted with brown, sepals to 3 in. long, petals to 1¾ in. long, lip whitish. Late spring. Colombia, Venezuela.

Lanceana Lindl. [*B. pumila* Lindl.]. Pseudobulbs strongly flattened, 1–3-lvd.; lvs. lanceolate-oblong, to 12 in. long; infl. longer than lvs., densely many-fld.; sepals and petals yellow, with brown markings, sepals 2¼ in. long, petals 1½ in. long, lip oblong-pandurate, yellowish-white, flecked with brown, acute, with a pair of white calluses at base. Surinam, Venezuela.

Lawrenceana Lindl. Pseudobulbs over 2 in. long, 2-lvd.; lvs. oblong or lanceolate, to 8 in. long; infl. to 2 ft. long or more; sepals and petals greenish- or bright yellow, spotted with brown, sepals almost 3 in. long, petals 1¼ in. long, lip light yellow, without flecks. Late spring. Guyana, Surinam. Var. longissima: *B. longissima*.

longissima (Rchb.f.) Nash [*B. Lawrenceana* var. *longissima* Rchb.f.]. Pseudobulbs to 5 in. long, 1- or 2-lvd.; lvs. to 15 in. long and 2¼ in. wide; infl. to 2 ft. long, 10–15-fld.; sepals and petals golden-yellow or greenish-yellow, spotted with brown at base, lateral sepals to 12 in. long, petals about 3 in. long, lip acuminate, pale yellow or white, spotted with red-brown. Late winter–autumn. Costa Rica.

maculata R. Br. [*B. guttata* Lindl.]. Differs from *B. longissima* in having lateral sepals only 2–3 in. long, petals smaller, and lip much broader, acute. Spring–summer, autumn. W. Indies and Cent. Amer.

pumila: *B. Lanceana*.

verrucosa Batem. [*B. brachiata* Lindl.]. Pseudobulbs to 3 in. long or more, 2-lvd.; lvs. oblong or elliptic-oblong, to 1 ft. long; infl. to about 2¼ ft. long, 4–16-fld.; sepals and petals green or yellowish, spotted with brown at base, sepals 3–5 in. long, petals to 2 in. long, lip white, warty, spotted with dark green toward base. Spring–early summer. Mex., Guatemala, Honduras, Venezuela.

BRASSICA L. [*Sinapis* L.]. COLE, MUSTARD. *Cruciferae.* Probably more than 40 spp. of mostly ann., bien., or sometimes per. herbs or small shrubs of Old World origin, but the nativity of many unknown; plants erect, tall, branched, and for the most part glabrous, often glaucous; lower lvs. variously pinnatifid or lyrate or strongly toothed; fls. in terminal racemes, yellow, yellowish-white or sometimes white, sepals 4, petals 4, clawed, lateral nectaries prismatic, deep green; fr. an elongate silique, valves convex, with prominent mid-vein.

acephala: *B. oleracea*, Acephala Group.

alba: *B. hirta*.

alboglabra: *B. oleracea*, Alboglabra Group.

arvensis: *B. Kaber*.

botrytis: *B. oleracea*, Botrytis Group.

bullata: see *B. oleracea*, Capitata Group.

campestris: *B. Rapa*.

capitata: *B. oleracea*, Capitata Group.

cauliflora: *B. oleracea*, Botrytis Group.

caulorapa: *B. oleracea*, Gongylodes Group.

chinensis: *B. Rapa*, Chinensis Group.

fimbriata: *B. Napus*, Pabularia Group.

gemmifera: *B. oleracea*, Gemmifera Group.

hirta Moench [*B. alba* (L.) Rabenh., not Gilib.; *Sinapis alba* L.]. WHITE M. Ann., to 4 ft., sparsely hairy; lvs. elliptic to obovate, deeply divided at the sides; fls. yellow, about ½ in. long; siliques spreading, to 1½ in. long, lower part seed-bearing and noduloso, beak flat. Medit. region, w. Asia; naturalized in N. Amer. Cult. for its mustard- and oil-producing seeds, also for greens.

japonica: *B. juncea* var.

juncea (L.) Czernisk. [*B. rugosa* Hort.; *Sinapis juncea* L.]. BROWN M., INDIAN M., LEAF M., MUSTARD GREENS. Ann., to 4 ft., green but st. sometimes slightly glaucous; lower lvs. elliptic to obovate, lyrate-lobed or divided, toothed or scalloped, rather thin, st.-lvs. narrowed at base but not clasping; fls. bright yellow; siliques to 1½ in. long. Eur., Asia. Much cult. for spring greens and as an oilseed, also spontaneous and a weed in N. Amer. Var. crispifolia L. H. Bailey [*B. japonica* Hort., not Thunb.]. CURLED M., SOUTHERN C. M., OSTRICH-PLUME. Lvs. cut, curled, crisped. The commonest leaf mustard for greens. Var. foliosa L. H. Bailey. BROAD-LEAVED M. Lvs. very large. Grown for greens. Var. longidens L. H. Bailey. Lvs. long, narrow, with large, prongliike teeth. Var. multisecta L. H. Bailey. Lvs. finely divided. See *Mustard*.

Kaber (DC.) Wheeler [*B. arvensis* (L.) Rabenh., not L.; *Sinapis arvensis* L.; *S. Kaber* DC.]. CHARLOCK, CALIFORNIA RAPE. Ann., to 3 ft. or more, green, commonly hispid toward base and sometimes above; lvs. ovate to oblong-ovate, variously lobed or lyrate, not clasping; fls. yellow, small; siliques about ¾ in. long or less, noduloso, beak often ½ in. long or more. Probably native in Medit. region. Sometimes cult. for mustard, but seeds not pungent; an early-flowering weed of waste places and grain fields.

Napobrassica: *B. Napus*, Napobrassica Group.

Napus L. RAPE. COLZA. Ann., but late-sown plants overwintering and flowering the following spring, making thin taproot; lvs. glaucous, lower lvs. lyrate-pinnatifid, sparsely bristly, petioled, middle and upper lvs. oblong-lanceolate, thick, clasping and sessile; fls. pale yellow; siliques to 4¼ in. long, ascending, on rather slender pedicels, beak to 1 in. long. Nativity unknown. In N. Amer. sown late as a forage and cover crop for late autumn and early spring. Elsewhere ann. or summer races of rape are grown for the seed, used for oil and as birdseed. See *Rapa*.

Napobrassica Group (*B. Napobrassica* Mill.; *B. Napobrassica* var. *solidiflora* L. H. Bailey). RUTABAGA, SWEDE, SWEDISH TURNIP. Thickened root with solid yellow or white flesh and with long neck or crown often withstanding winter in the North; siliques much spreading, on short pedicels, the beak short, stout. See *Rutabaga*.

Pabularia Group (*B. fimbriata* (Mill.) DC., *B. oleracea* var. *fimbriata* Mill.). SIBERIAN KALE. HANOVER SALAD. Low, dwarf bien., producing much edible herbage for winter and spring use, then going to seed; lvs. oblong or narrower, deeply lobed at the sides, curled or fringed, glaucous-blue, sometimes purplish. See *Kale*.

narinosa L. H. Bailey. BROAD-BEAKED M. Stout, low, bien., glabrous, not glaucous; lower lvs. in short clusters, orbicular-ovate, small, mostly entire, puckered, petioles broad, white, st. lvs. very broad, entire, clasping; fls. yellow; siliques very thick, ¾ in. long or less, ⅛ or ⅙ as broad, beak very short, stout. Probably Ana. Grown as a potherb by Chinese.

nigra (L.) W. D. J. Koch (*Sinapis nigra* L.). BLACK M. Much-branched ann., to 6 ft. and more, mostly hispid-hairy at least below, green, little if at all glaucous; lvs. pinnatifid to lyrate, dentate, petioled; fls. yellow, in many short racemes; siliques appressed to rachis, 1 in. long or less, 4-sided. Eurasia. Widespread weed; cult. as a main source of pungent table mustard.

oleracea L. WILD CABBAGE. Stout ann. to per., sometimes bien., glabrous, glaucous; lvs. thick, lower lvs. rounded or obovate, to 20 in. long, lobed at base, st. lvs. narrow, long, sometimes clasping; fls. whitish-yellow or cream-yellow, to 1 in. long; siliques spreading, to 4 in. long, seeds large, round. Coastal, w. and s. Eur. Represented in cult. by many forms, including several common vegetables. All forms, herein assigned to groups, have similar cult. requirements, including a cool growing season and deep, fertile soil capable of holding abundant moisture. Var. fimbriata: *B. Napus*, Pabularia Group.

Acephala Group (var. *acephala* DC.; *B. acephala* of auth.). KALE, TALL K., CABBAGE K., TREE K., DECORATIVE K., FLOWERING K., KITCHEN K., ORNAMENTAL K., ORNAMENTAL-LEAVED K., SCOTCH M., FLOWERING CABBAGE, COW C., COLLARDS, COLE, COLEWORT, BORECOLE, BRASCHETTE. St. usually unbranched, lvs. separate or only in loose rosettes, not making solid heads, thick, glaucous. The kales are planted in late spring or in late summer to produce either an autumn or early spring crop. Where winters are mild the plants may stand for a year or more. The ornamental kale, with rosettes of variously colored white, pink, or purplish lvs., often with fringed margins, is similarly planted from seed to produce autumn or winter bedding plants. See *Collard* and *Kale*.

Alboglabra Group (var. *alboglabra* (L. H. Bailey) Musil; *B. alboglabra* L. H. Bailey). CHINESE KALE. Ann., sometimes overwintering, to 3 ft., glabrous, very glaucous; lvs. thick, lower lvs. elliptic, to 10 in. long, sinuate, upper st. lvs. long-oblong and petioled or at least not clasping; fls. white; siliques 2–3 in. long. Probably native to Asia, where grown as a potherb.

Botrytis Group (var. *botrytis* L.; *B. botrytis* (L.) Mill.; *B. cauliflora* Gars.). BROCCOLI, CAULIFLOWER. Low, with stout, short st.; infl. a dense, terminal head formed of thickened, modified fl. clusters overtopped by lvs. Cult. of cauliflower and broccoli is similar to that of cabbage, but the plants are more tender to frost and less tolerant of heat and dryness. Broccoli requires a longer growing season than cauliflower. See *Broccoli* and *Cauliflower*.

Capitata Group (var. *capitata* L.; *B. capitata* of auth.). CABBAGE, HEAD C. Low, with stout, short st., bearing dense, terminal head of lvs. In one form, the SAVOY CABBAGE (var. *bullata* DC.; *B. bullata* of auth.), the lvs. are blistered and puckered. Cvs. differ in season of maturity and in color, size, and shape of the head. In all stages of development they withstand considerable frost, although young plants from hotbeds must be hardened off. See *Cabbage*.

Gemmifera Group (var. *gemmifera* Zenk.; *B. gemmifera* of auth.). SPROUTS, BRUSSELS S. St. simple, erect, to 3 ft., with small, compact, edible buds. See *Brussels sprouts*.

Gongylodes Group (*B. caulorapa* Pasq.). KOHLRABI. Low, stout bien., st. enlarging just above ground into a turniplike, edible tuber; lvs. elliptic, 10 in. long or less, long-petioled; fls. cream-yellow; siliques 2–3 in. long, beak short, thick. There are green- and purplish-stemmed cvs. Cult. as for turnips. Tubers should be harvested when 2 or 3 in. in diam. See *Kohlrabi*.

Italica Group (var. *italica* Plenck). ITALIAN BROCCOLI, ASPARAGUS B., SPROUTING B. Differs from Botrytis Group in the fl. brs. thickened, but not condensed into a solid head.

Tronchuda Group (var. *Tronchuda* L. H. Bailey). TRONCHUDA KALE, PORTUGUESE K., TRONCHUDA CABBAGE, PORTUGUESE C. Low, cabbagelike plant, without compact heads of lvs., with fleshy petiole and broad midribs. Lvs. used much like celery.

parachinensis L. H. Bailey. FALSE PAK-CHOI. Like *B. Rapa*, Chinese Group, but with basal lvs. more nearly orbicular, petiole not margined, and st. lvs. narrowed to base, not clasping. Probably e. Asia. Grown by Chinese as a potherb.

pekinensis: *B. Rapa*, Pekinensis Group.

perviridis: *B. Rapa*, Perviridis Group.

Rapa L. (*B. campestris* L.). FIELD M. Ann. or bien., root flat or globose, without a long neck or crown; lvs. lyrate-pinnatifid, to 20 in. long, soft but hispid, clasping; fls. yellow; siliques 2½ in. long or less. Eur. Var. lorifolia: Rapifera Group. Var. septiceps: Rapifera Group. See *Mustard*.

Chinensis Group (*B. chinensis* L.). PAK-CHOI, CELERY MUSTARD CHINESE M. Ann. or bien., glabrous, somewhat glaucous at maturity; lower lvs. glossy, making a rather compact cluster to 20 in. high, but not a head, obovate, entire or nearly so, petiole thickened, succulent, white, narrowly winged or margined but not jagged, st. lvs. clasping; fls. pale yellow, ¾ in. long; siliques to 2½ in. long. In habit of growth resembling garden celery or chard. Much cult. in Asia for its succulent lvs.

Pekinensis Group (*B. pekinensis* (Lour.) Rupr.). PE-TSAI, CHINESE CABBAGE, CELERY C. Ann., glabrous or essentially so; lvs. soft, green, basal lvs. large, very broad, undulate or obscurely toothed, petiole broad, flat, with jagged wings, st. lvs. petioled or clasping; fls. light yellow; siliques 2½ in. long. Grown as a cool-season vegetable, the lvs. forming a more or less solid head.

Perviridis Group (var. *pervindis* L. H. Bailey; *B. perviridis* (L. H. Bailey) L. H. Bailey). TENDERGREEN, SPINACH M. Ann. or perhaps bien., to 6 ft. in fr., branching above; lower lvs. many, spatulate-oblong, nearly entire, glossy green, tender, petiole not lobed; seeds small, somewhat angled. Grown in N. Amer. for its edible foliage, but the thick, tuberous crown to 3 in. across, pickled in Asia.

Rapifera Group (var. *lorifolia* L. H. Bailey; var. *septiceps* L. H. Bailey; *B. septiceps* (L. H. Bailey) L. H. Bailey). TURNIP, SEVEN-TOP T., RAPINI. Stout bien., glaucous, very leafy and florferous, with several tall sts. from root crown; lower lvs. with few deep lobes, st. lvs. clasping; fls. small, in short clusters; seeds small, angled or irregular. One of the oldest root crops. Turnips are short-season plants for cool climates. The roots are many sizes and shapes, with white or yellow flesh. Growing shoots used as greens. For use as a salad plant it is usually sown in late summer and early autumn. Sometimes called BROCCOLI or ITALIAN KALE. See *Turnip*.

Ruvo Group (*B. Ruvo* L. H. Bailey). RUVO KALE, TURNIP BROCCOLI, ITALIAN TURNIP, BROCCOLI RAAB. Ann. if sown in spring, bien. if sown in autumn, 2½–3½ ft. at maturity, with taproot; lvs. lyrate-pinnatifid, with lobes on petioles, dark green, often glossy; fls. small, in close clusters; siliques small, about 2 in. long. Not to be confused with Italian broccoli, *B. oleracea*, Italica Group.

rugosa: *B. juncea*.

Ruvo: *B. Rapa*, Ruvo Group.

septiceps: *B. Rapa*, Rapifera Group.

BRASSICACEAE: see *CRUCIFERAE*.

BRASSIOPHOENIX Burret. *Palmae*. Two spp. of solitary, unarmed, monoecious palms of New Guinea; lvs. pinnate, sheaths tubular, forming a prominent crownshaft, pinnae cuneate, 3-pronged at apex with prominent midrib and marginal veins; infl. below lvs., somewhat long-peduncled, bracts 2, the upper protruding from the lower in bud, rachillae with fls. in triads (2 male and 1 female); male fls. symmetrical, sepals 3, imbricate, petals 3, valvate, stamens many, anthers attached by base, pistillode shorter than the stamens, female fls. with sepals and petals imbricate, staminodes about 6, small, dentiform, pistil ovoid, 1-celled, 1-ovuled; fr. ovoid with terminal stigmatic residue, scarlet or yellowish-orange, endocarp hard, 3- or 9-ribbed, seed 3-sulcate, endosperm homogeneous.

For culture see *Palms*.

Schumannii (Becc.) Essig. To 30 ft.; lvs. 5–9 ft. long, rachis conspicuously dark-scaly, pinnae 9–10 on each side; infl. stout, few-branched, dark scaly; fls. cream-colored, male buds ⅜–½ in. long; fr. yellowish-orange, 1½–1¾ in. long.

long, petals cream-colored, 5/16 in. long. Mex. Var. **Stansburiana** (Torr.) Jeps. Bark grayish. lf. blades toothed. E. Calif. to Colo. and New Mex. Zone 6.

COXELLA: *ACIPHYLLA*.

CRABAPPLE: see *Apple*.

CRAIBIA Harms & S. T. Dunn. *Leguminosae* (subfamily *Faboideae*. About 10 spp. of trees and shrubs of trop. Afr.; lvs. alt., odd-pinnate or with 1 lft., the lfts. alt.; fls. racemose or panicled, often white, upper 2 calyx lobes united, stamens 10, 9 united and 1 free; fr. an ovate to oblong-obovate, flat legume, early dehiscent, seeds 1–2.

Brownii S. T. Dunn (*C. Elliotii* S. T. Dunn). Slow-growing tree, to 40 ft., with large spreading crown; lfts. 3–7, elliptic, to 4 in. long, acuminate; fls. very fragrant, white or tinged pink, in terminal racemes to 3 in. long; fr. obovate, to 2¾ in. long, narrowed at each end, 1-seeded. Kenya.

Elliotii: *C. Brownii*.

CRAMBE L. *Cruciferae*. About 20 spp. of ann. to per. herbs, mostly from Canary Is. to w. Asia, sometimes woody at base, glabrous or with unbranched hairs; lvs. mostly thick or fleshy, glaucous, often very large, lobed, cut, lyrate, or pinnatifid; fls. small, many, in racemes or panicles, sepals 4, petals 4, white, with a short claw or wedge-shaped basally; fr. a 2-jointed indehiscent silicle, the upper joint 1-seeded and globular.

cordifolia Steven. COLEWORT. Stout per., to 7 ft.; basal lvs. cordate, to 2 ft. across and more, somewhat lobed and coarsely toothed, more or less hispid-hairy, long-petioled; fls. 5/16 in. across, in a great, terminal, leafless panicle. Caucasus. Grown as an ornamental because of its striking appearance.

maritima L. SEA KALE, SCURVY GRASS. Stout, stocky per., to 3 ft.; lvs. large, glaucous-blue, fleshy, brittle, basal lvs. to 2 ft. long or more and nearly as broad, notched and shallowly lobed, stout-petioled; fls. in panicles. Seacoasts, w. Eur. to Asia Minor. Grown for succulent spring shoots, which are blanched. See *Sea Kale*.

hispanica L. Slender ann., to 3 ft., usually densely hispid; lvs. with elliptic to nearly orbicular terminal segm., lobed or lyrate below, to 3 in. across, sinuate; fls. in long open racemes. Medit. region. Cult. as a commercial oilseed crop.

CRANBERRY. Native to North America, the cranberry, *Vaccinium macrocarpon*, is cultivated entirely in the United States and Canada. Leading states producing cranberries in developed bogs or swamps are Massachusetts, Wisconsin, New Jersey, Washington, and Oregon. Canada has a limited acreage in British Columbia, Quebec, and parts of Nova Scotia. The small or European cranberry, *Vaccinium Oxycoccos*, native in the northern parts of America, is not cultivated. The fruits of the mountain cranberry or lingenberry, *Vaccinium Vitis-idaea*, are often collected from the wild and marketed, especially in Europe.

The cranberry plant is a low-growing vine with persistent leaves and a shallow, fine, fibrous root system. In late summer flower buds are initiated near the end of shoots (uprights) that arise from the main runners and on which the fruit are borne the subsequent year. Adequate pollination is essential and bee colonies are generally brought into bogs during the flowering period.

The cranberry is restricted to acid soils of pH 3.2 to 4.5; alkaline peat and ordinary garden and farm soils are not suitable for its culture. A large supply of water is needed near the bogs for irrigation, as well as for flooding as a means of protection against winter injury, untimely frosts, and insects. All except the West Coast bogs need to be flooded in winter to prevent "winter killing," a grower's term for winter dessication, a killing of the plants caused by moisture loss from the leaves at a time when roots are in frozen ground. It is unnecessary and undesirable to flood higher than the tallest cranberry vines.

Because cranberry bogs are situated in the lower elevations of the landscape, they are more susceptible than most crops to frosts in spring and autumn, when, on clear, still nights, the heavier, cold air from surrounding uplands drains onto the bogs and stratifies, with the coldest layers at the base and warmer air above. Formerly it was customary to flood the bogs in anticipation of hazardous low temperatures, but since the mid-1960's some two-thirds of the cranberry acreage have been provided with low-gallonage sprinkler systems which provide almost instantaneous frost protection. Despite the development of sophisticated frost-warning systems, flooding the bogs is at best slow, requiring about 300,000 gallons per acre, and the onset of low temperatures frequently outpaced the protecting flood. With sprinklers, protection is assured with completion of the first rotation of the sprinkler heads, and protection continues as long as they are in operation.

Sprinklers are much more economical of water, most being designed to use about 50 gallons per acre per minute, an acre-inch being needed for all-night frost protection. They are infinitely more useful for summer irrigation than flooding and they have proved themselves efficient in the distribution of fungicides and insecticides.

New commercial bogs are developed in a series of beds about two acres in size and serviced by a single reservoir of water. The preparation and planting of a cranberry bog is an expensive and time-consuming operation. Existing vegetation and tree stumps must be removed and the peat leveled. Ditches must be dug around the swamp and at intervals through the bog to facilitate flooding and drainage. In most areas a few inches of sand is spread on the peat before spreading newly mown cuttings 3–4 inches long over the surface and "discing-in" with a simple machine looking like a disc harrow but with flat, blunt blades.

Rooted cuttings may also be planted at 12-inch spacings in and between rows. Then follows three or four years in which weed and insect control must be achieved before the first commercial harvest can be made. Every three to five years bogs are sanded with ¼ inch of sand in the autumn after harvest to provide a suitable medium for root growth and insect control. During the growing season the water table is maintained at 9–12 inches below the surface. With careful management, a cranberry bog may continue to produce an annual crop thereafter for a century or more.

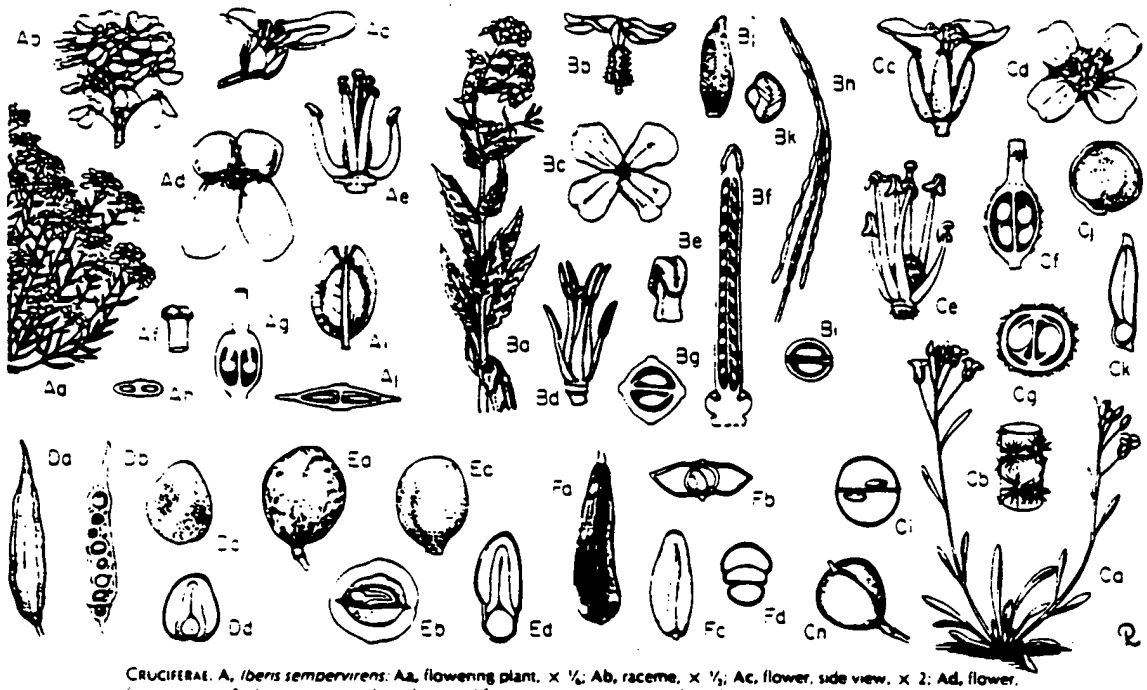
Because of high labor costs and a short harvesting season, cranberries are now largely harvested with special machines. Water harvesting, where the bog is flooded to a depth of 6–8 inches, is preferred over dry harvesting. The harvester rakes or beats the berries from the vines. These berries float to the surface and are gathered. Dry harvesting by mechanical means is less efficient because up to 30 percent of the crop is lost by berries dropping to the surface of the bog. Cranberries are now seldom harvested with hand scoops or by hand except occasionally for finishing up the margins of the bogs where it is difficult for machines to operate.

The average yield of an acre of cranberries is 100 barrels and occasional bogs will produce double that. Over half of all cranberries grown for processing are used in cranberry juices; the balance is made into sauces, relishes, and pie fillings. Only 20 percent of the cranberry crop is sold as fresh fruit.

Most of the annual crop is derived from named cultivars representing selections from wild cranberry vines. 'Early Black' and 'Howes' predominate in Massachusetts and New Jersey, 'Searless Jumbo' in Wisconsin, and 'McFarlin' in the Pacific Northwest. Hybrids derived from crosses of named selections are slowly being introduced, 'Stevens' being notable, particularly in Wisconsin.

The raising of cranberries is a highly specialized form of agriculture requiring heavy capital investment, daily surveillance of weather, insects, and other hazards, and in recent years the margin of profit has been narrow.

CRASPEDIA G. Forst. *Compositae* (Inula Tribe). About 7 spp. of ann. or per. herbs, native to Australia, New Zeal., and Tasmania; lvs. in a basal rosette or alt. on sts., entire; infl. a compound head composed of many 3–10-fld. individual heads crowded together in an ovoid or globose terminal clus-



CRUCIFERAE. A, *Iberis sempervirens*: Aa, flowering plant, × ½; Ab, raceme, × ⅓; Ac, flower, side view, × 2; Ad, flower, face view, × 2; Ae, stamens and pistil, × 4; Af, stigma, × 6; Ag, base of pistil, vertical section, × 6; Ah, ovary, cross section, × 6; Ai, fruit (a silicle), × 1; Aj, fruit, cross section, × 2. B, *Hesperis matronalis*: Ba, flowering stem, × ⅛; Bb, flower, side view, × ½; Bc, flower, face view, × ½; Bd, stamens and pistil, × 1½; Be, stigma, × 10; Bf, pistil, vertical section, × 5; Bg, ovary, cross section, × 15; Bh, fruit (a silique), × ½; Bi, fruit, cross section, × 2; Bj, seed, × 5; Bk, seed, cross section (cotyledons incumbent), × 6. C, *Lesquerella alpina*: Ca, flowering plant, × ½; Cb, segment of stem with stellate hairs, × 10; Cc, flower, side view, × 2; Cd, flower, face view, × 2; Ce, stamens and pistil, × 3; Cf, base of pistil, ovary in vertical section, × 6; Cg, ovary, cross section, × 10; Ch, fruit, × 1½; Ci, fruit, cross section, × 1½; Cj, seed, × 5; Ck, seed, cross section (cotyledons accumbent), × 8. D, *Raphanus sativus*: Da, fruit, × ½; Db, fruit, vertical section, × ½; Dc, seed, × 3; Dd, seed, cross section (cotyledons conduplicate), × 3. E, *Crambe maritima*: Ea, fruit, × 1½; Eb, fruit, cross section, × 1½; Ec, seed, × 2; Ed, seed, cross section (cotyledons conduplicate), × 3. F, *Isatis tinctoria*: Fa, fruit, × 1½; Fb, fruit, cross section, × 4; Fc, seed, × 5; Fd, seed, cross section (cotyledons incumbent), × 8.

of horticulture with variegated foliage belong to the genus *Codiaeum*. The following epithets sometimes, but incorrectly, used in *Croton* are properly treated as cultivars of *Codiaeum variegatum* var. *pictum*, and are listed under that name: *Andreanum, angustissimum, anietumense, aucubifolium, bogoriense, bruxellense, Craigii, delicatissimum, edmontonense, glonosum, gracioseum, Grusonii, interruptum, linearis nigrescens, montefontainense, pictum, punctatum, punctatum aureum, Reidii, Sanderi, Schattii, spirale, Warrenii, Weismannii.*

macrostachys Hochst. ex A. Rich. Monoecious tree, to 30 ft. or more; lvs. ovate, 1½–4 in. long, cordate, crenulate; racemes usually unisexual, male to 10 in. long, many-fld., female to 4 in. long; female fls. without petals, male fls. with petals, stamens 15. S.-cent., se. and e. Afr.

megalobotrys Müll. Arg. Large, dioecious tree, 20–30 ft.; lvs. ovate to ovate-lanceolate, 1½–3 in. long, long-acuminate, rounded or truncate, serrate to dentate; male fls. with petals, many, in 1–3 in. racemes on short lateral branchlets, stamens 20–25. Se. and s.-cent. Afr.

megalocarpus Hutch. Monoecious tree, 70–80 ft.; lvs. oblong-lanceolate or elliptic-oblong, to 5 in. long, entire, densely scaly below; fls. with petals, in racemes, to 10 in. long, female fls. below, male fls. above, stamens 25. Trop. Afr.

monanthogynus Michx. PRAIRIE TEA. Monoecious, glandular ann. to 2 ft., sts. often umbellately 3–4-forked in lower part; lvs. oblong to ovate, entire; male fls. with petals, stamens 3–8, female fls. without petals, ovary often with only 1 or 2 cells. Va. to Kans., s. to Ga., Tex., and Mex.

pictum. *Codiaeum variegatum* var.

CRUCIANELLA L. CROSSWORT. *Rubiaceae*. About 30 or more spp. of ann. or per. herbs or half-shrubs, with slender 4-angled sts., from Medit. region, w. to Iran and cent. Asia; upper lvs. opp., lacking stipules, lower lvs. whorled; fls. in spikes or clusters subtended by bracts, small, white, rosy or blue, 4–5-merous, corolla funnelform, tube long; fr. dry, dehiscent into 2 halves.

Grown in rock gardens, where they thrive in partial shade. Propagated by division and by seeds.

angustifolia L. Ann., to 1½ ft.; lvs. in whorls of 4–6, all linear-subulate, very scabrous, margins recurved; fls. white, minute. Cent. and s. Eur.

berbeoea Forssk. Ann., to 1 ft. or more; lvs. ovate-oblong or the upper linear; fls. in dense, linear spikes to 2 in. long. Egypt.

latifolia L. Ann., to 1½ ft.; lower lvs. obovate to oblong, upper lvs. linear-lanceolate; fls. whitish, in slender, linear spikes. S. Eur.

stylosa Trin. Prostrate ann., sts. to 9 in. long; lvs. in whorls of 8–9, lanceolate, to ¾ in. long; fls. deep rose, in globose heads ½ in. across, styles long-exserted. Iran. Cv. 'Carminea' is listed.

CRUCIFERAE Juss. or, alternatively, **BRASSICACEAE** Burnett. MUSTARD FAMILY. Dicot.; about 350 genera and 3,200 spp. of pungent or acrid herbs of various habit; lvs. alt., without stipules; fls. in terminal racemes or corymbs, usually bisexual, regular, sepals 4, deciduous, petals 4, their spreading limbs forming a cross, stamens 6, 2 of these shorter and inserted lower than the others, pistil of 2 carpels, ovary superior; fr. a 2-celled caps., varying in form (known as a silique when elongated or a silicle when short and broad) but usually opening by 2 valves from below, seeds without endosperm, filled by a large embryo curved or folded in various ways, yielding (along with the fr.) the important taxonomic characters of the family.

The cultivated genera are: *Aethionema, Alyssoides, Alyssum, Anastatica, Arabidopsis, Arabis, Armoracia, Aubrieta, Aurinia, Barbarea, Berteroa, Biscutella, Brassica, Bunias, Cardamine, Cheiranthus, Cochlearia, Crambe, Dentaria, Diplotaxis, Dithyrea, Draba, Eruca, Erysimum, Fibigia, Heliophila, Hesperis, Hugueninia, Hutchinsia, Iberis, Ionopsidium, Isatis, Kernera, Lepidium, Lesquerella, Lobularia,*

Lunaria, Malcolmia, Matthiola, Morisia, Nasturtium, Peltaria, Petrocallis, Phoenicaulis, Physaria, Raphanus, Roripa, Schivereckia, Schizopetalon, Sisymbrium, Smelowskia, Stanleya, Stenodraba, Subularia, Thelypodium, Thlaspi, and *Thysanocarpus.*

The mustard family includes many ornamental species. It is also the source of important vegetables, particularly in the genera *Brassica* broccoli, Brussels sprouts, cabbage, Chinese cabbage, kale, kohlrabi, rutabaga, turnip), *Lepidium* (cress), *Nasturtium* (watercress), and *Raphanus* (radish); of condiment-producing plants, such as the common horseradish (*Armoracia*), Japanese horseradish (*Wasabia japonica*), and mustard (*Brassica*), and of important oilseed plants (*Brassica, Crambe*)

CRUPINA Cass. *Compositae* (Carduus Tribe). Two or 3 spp. of ann. herbs, native to s. Eur. and w. Asia; lvs. pinnately dissected; fl. heads long-peduncled, involucral bracts acuminate, not spiny; fls. light purple, all tubular, few; pappus blackish-brown, in 2 rows, the outer of graduated bristles, the inner of scales.

vulgaris Cass. [*Centaurea Crupina* L.]. To 2 ft.; lowermost lvs. oblanceolate, to 6 in. long, entire or lobed, the rest smaller, cut into denticulate, linear segms.; heads ¾ in. long, 3–5-fld.; fls. not much longer than involucre. S. Eur.

CRUSEA Schlechtend. & Cham. *Rubiaceae.* About 13 spp. of usually low ann. or per. herbs, native to s. Ariz. and New Mex., s. to Mex. and Cent. Amer., with sts. cylindrical or sometimes more or less 4-angled; lvs. 4-ranked, ovate or lanceolate, conspicuously nerved, stipules united with petioles to form a sheath; fls. in heads surrounded by leaflike bracts, usually pink or violet, 4-merous, corolla funnelform, tube slender, lobes spreading; fr. longitudinally dehiscent into 2 dry, 1-seeded sections.

calocephala DC. [*C. violacea* Brongn. ex J. Neumann]. Per. or rarely ann., sts. usually decumbent, often rooting, cylindrical, hairy, erect sts. 4–10 in. or more; lvs. sessile, ovate, ¾–3¼ in. long, with 6 veins parallel to midvein, stipules awned; fls. violet. Mex. and Cent. Amer.

violacea: *C. calocephala.*

CRYOPHYTUM: *MESEMBRYANTHEMUM.*

CRYOSOPHILA Blume [*Acanthorrhiza* H. Wendl.]. *Palmae.* A few spp. of small or medium palms with bisexual fls., in Mex., Cent. Amer. and n. S. Amer., root-spines closely covering the trunk at least towards the base; lvs. palmate, divided nearly to base at center and then into 1- to several-ribbed, acute segms. on each side, petiole with smooth margins; infl. among lvs., arched, with tomentose loosely sheathing bracts on peduncle, rachillae many; fls. solitary, creamy-white to purplish, sepals 3, briefly united basally, petals 3, imbricate, stamens 6, filaments united to the middle or above, carpels 3, with subulate styles; fr. yellowish to white, globose to pear-shaped or oblong, seed globose with homogeneous endosperm and no intrusion of the seed coat.

Several species planted as ornamentals. For culture see *Palms.*

argentea Bartlett. To nearly 20 ft.; lvs. to 3 ft. long, ribs about 44, blade divided to middle and into about 9 segms. on each side, lacking conspicuous cross-veinlets when dry; fr. about ½ in. in diam. Brit. Honduras, Guatemala.

nana (HBK) Blume ex Salomon [*Acanthorrhiza aculeata* (Lieben.) H. Wendl.]. Small tree to 15 ft., trunk gray with short spines to 1 in. long; lvs. green above, silvery beneath, lacking conspicuous cross-veinlets when dry, ribs to 50, segms. to 2 ft. long, 1 in. wide; infl. short, to 1 ft. long in fr., lower bracts to 6 in. long; stamens united nearly to apex; fr. globose, to ⅝ in. long. Calcareous mts., w. Mex. Warmest parts of Zone 9b in Fla. Sometimes confused with *C. Warscewiczii.*

Warscewiczii (H. Wendl.) Bartlett. To 20 ft. or more, trunk gray with long root-spines at base, becoming nearly smooth above in age; lvs. green above, silvery beneath, with conspicuous cross-veinlets when dry, segms. 50–60, to 3 ft. long, 1¼ in. wide, 2–4-ribbed; infl. to 2 ft. long or more, lower bracts 8–10 in. long; stamens united about half their length; fr. globose to pear-shaped, to 1 in. long, white. Costa Rica to Panama. Cult. in s. Fla., often under the name *Acanthorrhiza aculeata.*

CRYPTANTHA Lehm. ex Fisch. & C. A. Mey. WHITE FORGET-ME-NOT. *Boraginaceae.* About 100 spp. of hispid or setose, ann. and per. herbs, mostly of w. N. Amer., but some native to w. and s. S. Amer.; lvs. simple, alt., entire; fls. white or rarely yellow, small, many, in bractless or bracted scorpioid spikes or racemes, rarely somewhat cymose-paniculate, calyx 5-lobed or -cleft, corolla 5-lobed, funnelform, corolla throat with scales, stamens 5, included; fr. of 1–4, erect, rough or smooth nutlets.

Rarely sown in the wild garden in the region where they grow.

intermedia (A. Gray) Greene. Much-branched, hispid ann., 1–1½ ft.; lvs. linear to lanceolate, to 1 in. long; infl. bractless; fls. white, to ⅛ in. across; nutlets usually 4, rough. N. Calif. to Baja Calif.

Sheldonii (Brand) Payson. Hairy per. with 1 or more ascending sts., to 10 in.; basal lvs. spatulate to oblanceolate, to 1¼ in. long; infl. bracted, with many yellow hairs; fls. white, to ⅛ in. wide; nutlets 4, somewhat glossy, rough. E. Wash. and Ore. to Montana.

CRYPTANTHUS Klotzsch. EARTH-STAR. *Bromeliaceae.* About 20 spp. of terrestrial stoloniferous herbs, native to Brazil, usually with flattened rosettes of lvs., rarely with leafy sts.; lvs. stiff, prickly-margined; fls. white or greenish-white, borne in small heads among the foliage, inner fls. often sterile, sepals united into a tube, petals not appendaged, united into a tube, the lobes spreading, ovary inferior; fr. a berry, seeds without appendages.

Grown as foliage plants under glass, in the home, or outdoors in warm climates. Propagated by offsets; thrive in bright sun or filtered shade. For culture see *Bromeliaceae.*

Hybrids of several spp. are listed, the most common parents being *C. bahianus, C. Beuckeri,* and *C. zonatus. Cryptanthus Beuckeri* and *C. bahianus* have been crossed with spp. of *Billbergia* and the resulting hybrids are listed as × *Cryptbergia* or sometimes as × *Biltanthus.*

acaulis (Lindl.) Beer. STARFISH PLANT. Nearly stemless; lvs. elliptic-lanceolate, to 6 in. long, 1¼ in. wide, with undulate, prickly margins, green, white-scurfy beneath; fls. 1⅝ in. long, tube of calyx much longer than the lobes, the lobes entire or nearly so. Small foliage plant with many cvs.: 'Roseo-pictus' is listed; 'Roseus', lvs. tinged rose-pink; 'Ruber' [var. *ruber* Hort. ex Beer], lvs. tinged red. Var. bromelioides *C. bromelioides.* Var. diversifolius: *C. diversifolius.*

bahianus L. B. Sm. St. long and leafy; lvs. narrowly triangular, to 10 in. long, ⅝ in. wide, with spines to ⅛ in. long, green and smooth above, becoming red-tinged, white-scurfy beneath; fls. to 1 in. long, petals white. Withstands full sun.

Bankeri: a listed name, probably in error for *C. Beuckeri.*

Beuckeri E. Morr. Lvs. to 5 in. long, 2 in. wide, narrowed to a petiole about 2 in. long, or the inner sessile and triangular, brownish-green or rosy-spotted, or striped with light green.

bivittatus (Hook.) Regel. Stemless or nearly so; lvs. strongly acuminate, arching, spiny, greenish-brown above, with 2 reddish or pink longitudinal stripes; fls. white. Cv. 'Luddemanii', of larger size. Cv. 'Minor', is listed.

bromelioides Otto & A. Dietr. [*C. acaulis* var. *bromelioides* (Otto & A. Dietr.) Mez]. PINK C. Lvs. all alike, not petioled, to 7 in. long, 1⅝ in. wide, spiny-margined, green above, silvery beneath; infl. on a scape 6 in. high; fls. many, in clusters of 4–6 in axils of keeled fl. bracts, milky-white, 1⅝ in. long, tube of calyx longer than the lobes. Sometimes grown under the name *C. terminalis.* Var. tricolor M. B. Foster. RAINBOW-STAR. Lvs. striped with ivory-white and green, overlaid with carmine-rose.

diversifolius Beer [*C. acaulis* var. *diversifolius* (Beer) Mez]. Lvs. dimorphic, gradually narrowed at the base, to 1 ft. long, 1¾ in. wide, uniformly colored above and beneath; fl. bracts to ⅝ in. long; sepals acuminate.

Fosterianus L. B. Sm. Lvs. constricted at base, all alike, thick and fleshy, marked with irregular dark brown crossbands above; calyx ⅛ in. long.

Lacerdae Ant. SILVER-STAR. Small, stemless; lvs. all alike, not petioled, scarcely more than 2 in. long, ashy-white-scurfy but with 2 longitudinal, glabrous, green stripes; infl. few-fld.; fls. milky-white, ⅝ in. long.

× Osyanus hort. name for the hybrid, *C. Beuckeri* E. Morr. × *C. Lacerdae* Ant.

Racinae a listed name of no botanical standing.

roseo-pictus a listed name of no botanical standing; probably *C. acaulis* cv. or *C. bivittatus.*

roseus a listed name of no botanical standing; probably *C. acaulis* cv.

rubescens a listed name of no botanical standing.

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A major inducer of anticarcinogenic protective enzymes from broccoli: Isolation and elucidation of structure

(chemoprotection/enzyme induction/isothiocyanates/sulforaphane/quinone reductase)

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Contributed by Paul Talalay, December 19, 1991

ABSTRACT Consumption of vegetables, especially crucifers, reduces the risk of developing cancer. Although the mechanisms of this protection are unclear, feeding of vegetables induces enzymes of xenobiotic metabolism and thereby accelerates the metabolic disposal of xenobiotics. Induction of phase II detoxication enzymes, such as quinone reductase [NAD(P)H:(quinone-acceptor) oxidoreductase, EC 1.6.99.2] and glutathione *S*-transferases (EC 2.5.1.18) in rodent tissues affords protection against carcinogens and other toxic electrophiles. To determine whether enzyme induction is responsible for the protective properties of vegetables in humans requires isolation of enzyme inducers from these sources. By monitoring quinone reductase induction in cultured murine hepatoma cells as the biological assay, we have isolated and identified (−)-1-isothiocyanato-(4*R*)-(methylsulfinyl)butane [CH_3—SO—$(CH_2)_4$—NCS, sulforaphane] as a major and very potent phase II enzyme inducer in SAGA broccoli (*Brassica oleracea italica*). Sulforaphane is a monofunctional inducer, like other anticarcinogenic isothiocyanates, and induces phase II enzymes selectively without the induction of aryl hydrocarbon receptor-dependent cytochromes P-450 (phase I enzymes). To elucidate the structural features responsible for the high inducer potency of sulforaphane, we synthesized racemic sulforaphane and analogues differing in the oxidation state of sulfur and the number of methylene groups: CH_3—SO_m—$(CH_2)_n$—NCS, where m = 0, 1, or 2 and n = 3, 4, or 5, and measured their inducer potencies in murine hepatoma cells. Sulforaphane is the most potent inducer, and the presence of oxygen on sulfur enhances potency. Sulforaphane and its sulfide and sulfone analogues induced both quinone reductase and glutathione transferase activities in several mouse tissues. The induction of detoxication enzymes by sulforaphane may be a significant component of the anticarcinogenic action of broccoli.

Individuals who consume large amounts of green and yellow vegetables have a lower risk of developing cancer (1–3). Feeding of such vegetables to rodents also protects against chemical carcinogenesis (4, 5), and it results in the induction in many tissues of phase II[§] enzymes—e.g., quinone reductase [QR: NAD(P)H:(quinone-acceptor) oxidoreductase, EC 1.6.99.2] and glutathione *S*-transferases (EC 2.5.1.18) (11–17). Although much evidence suggests that induction of these enzymes is a major mechanism responsible for this protection (18–20), the precise role of enzyme induction in protection of humans requires clarification. The preceding report (21) shows that measurement of QR activity in Hepa 1c1c7 murine hepatoma cells provides a rapid, reliable, and convenient index of phase II enzyme inducer activity in vegetables. Using this assay (21–24), we found that cruciferous vegetables (broccoli, cauliflower, mustard, cress, brussels sprouts) were a rich source of inducer activity. We chose to investigate broccoli (*Brassica oleracea italica*) specifically because it is consumed in substantial quantities by Western societies and has been shown to contain abundant phase II enzyme inducer activity (21). In this paper we describe the isolation and identification of a potent major phase II enzyme inducer from broccoli.

MATERIALS AND METHODS

Source of Vegetables and Preparation of Extracts. SAGA broccoli was grown by Andrew Ayer (Maine Packers, Caribou, ME). SAGA is synonymous with Mariner broccoli (Petoseed, Arroyo Grande, CA) and was adapted for growing in Maine by Smith, Ayer, Goughan, and Arrow. The broccoli was harvested when ripe, frozen immediately, shipped to our laboratory in dry ice, and stored at −20°C until processed.

For preliminary survey of inducer activity in broccoli samples, florets were homogenized with 2 vol of water at 4°C, and the resultant soups were lyophilized to give powders, which were stored at −20°C. Portions (400 mg) of these powders were extracted for 6 hr with 14 ml of acetonitrile in glass-stoppered vessels on a wrist-action shaker at 4°C. The extracts were filtered through a sintered glass funnel and evaporated to dryness in a rotating evaporator (<40°C). The residues were dissolved or suspended in 100 μl of dimethyl formamide and assayed for inducer activity.

Assay of Inducer Activity. Inducer potency for QR was measured in Hepa 1c1c7 murine hepatoma cells grown in 96-well microtiter plates (21, 24). The cells (10,000 per well) were grown for 24 hr and then exposed to inducer for 48 hr. Usually 20 μl of the solutions to be assayed (in acetonitrile or dimethyl formamide) was added to 10.0 ml of medium and 2-fold serial dilutions were used for the microtiter plates. The final organic solvent concentration was always less than 0.2% by volume. One unit of inducer activity is defined as the amount that when added to a single microtiter well (containing 150 μl of medium) doubles the QR specific activity. The inducer potency of compounds of known structure has been determined in the above system also, and it is expressed as

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Abbreviations: QR, quinone reductase [NAD(P)H:(quinone-acceptor) oxidoreductase, EC 1.6.99.2]; CD value, the concentration of a compound required to double the quinone reductase specific activity in Hepa 1c1c7 murine hepatoma cells.

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§Enzymes of xenobiotic metabolism belong to two families (6): (*i*) phase I enzymes (e.g., cytochromes P-450), which functionalize compounds, usually by oxidation or reduction; although their primary role is to detoxify xenobiotics, several cytochromes P-450 can activate procarcinogens to highly reactive ultimate carcinogens (7); and (*ii*) phase II enzymes, which conjugate functionalized products with endogenous ligands (e.g., glutathione, glucuronic acid, sulfate) and play primarily a detoxication role (8). QR is considered a phase II enzyme because it serves protective functions (9), is induced coordinately with other phase II enzymes, and is regulated by enhancer elements similar to those that regulate glutathione transferases (10).

the concentration required to double (CD value) the QR activity.

The inductions of QR and glutathione transferase activities in mouse organs were studied according to a standard protocol (25).

Synthesis of Compounds. (*R,S*)-Sulforaphane (CAS 4478-93-7) was prepared according to Schmid and Karrer (26) except that gaseous thiomethanol was replaced by sodium thiomethoxide. The sulfide analogues, $CH_3—S—(CH_2)_n—NCS$, where *n* is 4 [erucin (CAS 4430-36-8)] or 5 [berteroin (CAS 4430-42-6)] were prepared as described (27), and the three-carbon analogue [iberverin (CAS 505-79-3)] was prepared from phthalimidopropyl bromide (26). IR spectra of all three sulfide analogues showed strong absorptions near 2150 cm^{-1}, characteristic of isothiocyanates. ^1H NMR spectra of these compounds show sharp singlets at δ 2.10 ppm ($CH_3—S$ group). The sulfoxide analogues where *n* is 3 [iberin (CAS 505-44-2)] or 5 [alyssin (CAS 646-23-1)] were prepared by the same method as sulforaphane. IR spectra of these compounds showed strong absorptions near 2100 cm^{-1}, assigned to the —NCS group. ^1H NMR spectra also showed sharp singlets around δ 2.5 ppm, consistent with the presence of the $CH_3—SO$ group. The sulfone analogues, $CH_3—SO_2—(CH_2)_n—NCS$, where *n* is 3 [cheirolin (CAS 505-34-0)], 4 [erysolin (CAS 504-84-7)], or 5 (unreported) were prepared by known methods (28). ^1H NMR (δ = 2.9 ppm, for $CH_3—SO_2—$) and IR spectra of these compounds were consistent with the structures. Every analogue except 1-isothiocyanato-5-methylsulfonylpentane [$CH_3—SO_2—(CH_2)_5—NCS$] has been isolated from plants (29).

RESULTS

Isolation of Inducer Activity. We selected SAGA broccoli for study because acetonitrile extracts of lyophilized homogenates of this variety were especially rich in inducer activity (62,500 units per g) in comparison with other vegetables (21). Fractionation of acetonitrile extracts of SAGA broccoli by preparative reverse-phase HPLC (Fig. 1) with a water/methanol solvent gradient resulted in recovery of 70–90% of the applied inducer activity in the chromatographic fractions. Surprisingly, the majority (about 65–80% in several chromatographies) of the recovered activity was associated with a single and relatively sharp peak [fractions 18–23; eluted at 64–71% (vol/vol) methanol]. This HPLC procedure was therefore adopted as the first step of the larger-scale isolation of inducer activity.

Lyophilized SAGA broccoli was extracted three times with acetonitrile (35 ml/g) for 6 hr each at 4°C. The pooled extracts were filtered and evaporated to dryness under reduced pressure on a rotating evaporator (<40°C). About 1 g of residue from 640 g of fresh broccoli (64 g of lyophilized powder) contained 3.6×10^6 units of inducer activity. The residue was mixed thoroughly with 120 ml of methanol/water (25/75, vol/vol) and the insoluble fraction was discarded. Although not all of the residue obtained from the extraction was soluble in aqueous methanol, the solvent partition procedure resulted in substantial purification without significant loss of inducer activity. Portions of the extract were dried in a vacuum centrifuge and dissolved in small volumes of dimethyl formamide (0.75–1.0 ml per 50 mg of residue), and 50-mg portions were subjected to HPLC (nine runs) as described in the legend of Fig. 1. Fractions 18–23 from all runs were pooled, evaporated to dryness, applied in acetonitrile to five preparative silica TLC plates (100 × 200 × 0.25 mm), and developed with acetonitrile, which was run to the top of each plate three times. Four major fluorescence-quenching components were resolved, and nearly all (99%) of the inducer activity migrated at R_f 0.4. The active bands were eluted with acetonitrile, pooled, and fractionated by two runs on a second preparative reverse-phase HPLC in a water/

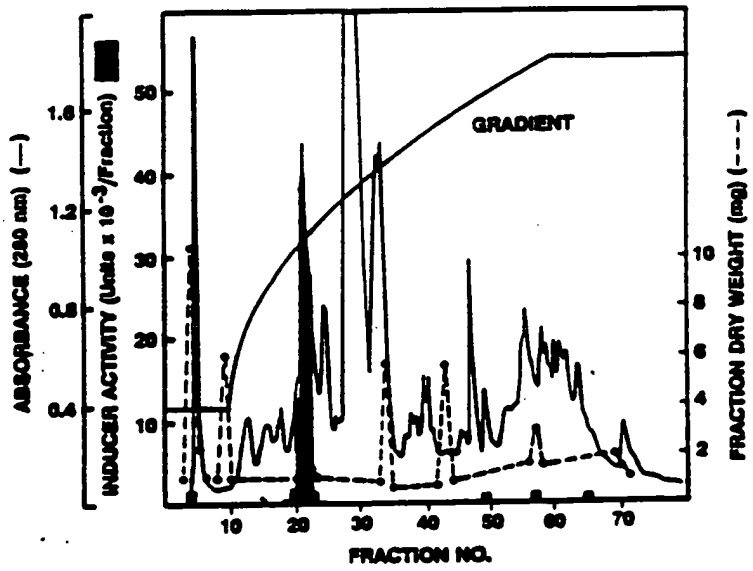


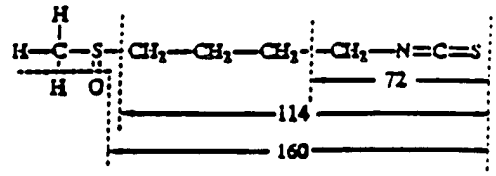
FIG. 1. Reverse-phase HPLC of acetonitrile extract of SAGA broccoli showing the distribution of absorbance at 280 nm, total inducer activity (units per fraction), and dry weight of each fraction. Lyophilized SAGA broccoli floret powder (16 g) was extracted three times (for 6 hr each) with 560-ml portions of acetonitrile on a shaker at 4°C. The extracts were filtered and evaporated to dryness on a rotating evaporator (<40°C). The residue (202 mg) was suspended in 3.0 ml of methanol and filtered successively through 0.45- and 0.22-μm porosity filters. The insoluble material was discarded. The filtrate was assayed for total inducer activity, and a 0.75-ml (50.5-mg) aliquot of the methanol extract was subjected to HPLC on a reverse-phase preparative column (Whatman; Partisil 10 ODS-2; 50 × 1.0 cm) equilibrated with methanol/water (30/70, vol/vol), eluted at a rate of 3.0 ml/min, and 6.0-ml fractions were collected. Elution solvent: 30 ml of initial solvent, followed by 330 ml of a convex gradient (Waters Gradient program 5) to 100% methanol, and then by 90 ml of 100% methanol. The fractions were evaporated on a vacuum centrifuge (Savant Speed-Vac Concentrator), and the residues were weighed, redissolved in 0.1 ml of dimethyl formamide, and assayed for inducer activity. The activity applied (0.75 ml = 104,000 units) was recovered principally in fractions 18–23 (84,600 units, 81%), and minor amounts of activity were found in fractions 4, 49, 57, and 65. The total recovery of inducer activity in all fractions was 90% of that applied to the column.

acetonitrile gradient (Fig. 2). Ultraviolet absorption and inducer activity were eluted in a sharp coincident peak (at 66% acetonitrile) that contained all of the activity applied to the column. Evaporation (<40°C) of the active fractions gave 8.9 mg of a slightly yellow liquid, which contained 558,000 inducer units (overall yield 15%) and migrated as a single band on TLC.

Identification of Inducer. The identity of the inducer was established by spectroscopic methods and confirmed by chemical synthesis. It is (−)-1-isothiocyanato-(4*R*)-(methylsulfinyl)butane, known as sulforaphane or sulphoraphane (CAS 4478-93-7):

Sulforaphane has been synthesized (26) and isolated from leaves of hoary cress (30) and from other plants (31), and the absolute configuration has been assigned (32). The closely related olefin sulforaphene [4-isothiocyanato-(1*R*)-(methylsulfinyl)-1-(*E*)-butene (CAS 2404-46-8)] has been isolated from radish seeds and other plants (33, 34) and has also been synthesized (35, 36).

The following evidence establishes that (*R*)-sulforaphane is the inducer isolated from broccoli. UV spectrum (H_2O): λ_{max} 238 nm, ε_{238} 910 $M^{-1}\cdot cm^{-1}$; addition of NaOH (0.1 M) blue-shifted (λ_{max} 226 nm) and intensified (ε_{226} 15,300 $M^{-1}\cdot cm^{-1}$) this absorption band, consistent with the behavior of isothiocyanates (37). IR (Fourier transform, neat): strong absorptions at 2179 and 2108 cm^{-1} and also at 1350 cm^{-1}, characteristic of isothiocyanates (27). ^1H NMR (400 MHz, C^2HCl_3): δ 3.60 (t, 2H, J = 6.1 Hz, $—CH_2—NCS$), 2.80–2.66 (m, 2H, $—CH_2—SO—$), 2.60 (s, 3H, $CH_3—SO—$), and 1.99–1.86 ppm (m, 4H, $—CH_2CH_2—$). ^{13}C NMR (400 MHz, C^2HCl_3): δ 53.5, 44.6, 38.7, 29.0, and 20.1 ppm. Mass spectrometry (fast atom bombardment; thioglycerol matrix) gave prominent peaks at 178 $(M + H)^+$ and 355 $(M_2 + H)^+$. Electron impact mass spectrometry gave a small molecular ion (M^+) at 177, and chemical ionization mass spectrometry gave a small molecular ion $(M + H)^+$ at 178 and prominent fragment ions with masses of 160, 114, and 72, consistent with the following fragmentation:



Precise masses of molecular and fragment ions obtained by electron impact mass spectrometry were 177.0286 (calculated for $C_6H_{11}NOS_2$, 177.0283), 160.0257 (calculated for $C_6H_{10}NS_2$, 160.0255), and 71.9909 (calculated for $C_2H_2NS_1$, 71.9908). In addition, for the mass 160 fragment, the peaks at 161 (M + 1) and 162 (M + 2) were 8.43% (calculated, 8.44%) and 9.45% (calculated, 10.2%), respectively, of the parent ion. Similarly, for the mass 72 fragment, the peaks at 73 (M + 1) and 74 (M + 2) were 3.42% (calculated, 3.32%) and 5.23% (calculated, 4.44%), respectively, of the parent ion. Hence the isotope compositions corrected for the natural isotope abundance (of ^{13}C, ^{15}N, ^{33}S, and ^{34}S) were consistent with the relative intensities of the M + 1 and M + 2 ions of both fragments. The optical rotation of the isolated material was $[\alpha]_D^{23}$ −63.6° (c = 0.5, CH_2Cl_2), thus establishing that the product is largely, if not exclusively, the (−)-(*R*) enantiomer (literature $[\alpha]_D$ −79°, −73.2°, −66°; refs. 26, 30, and 38, respectively). The spectroscopic properties of synthetic (*R,S*)-sulforaphane were identical to those of the isolated product.

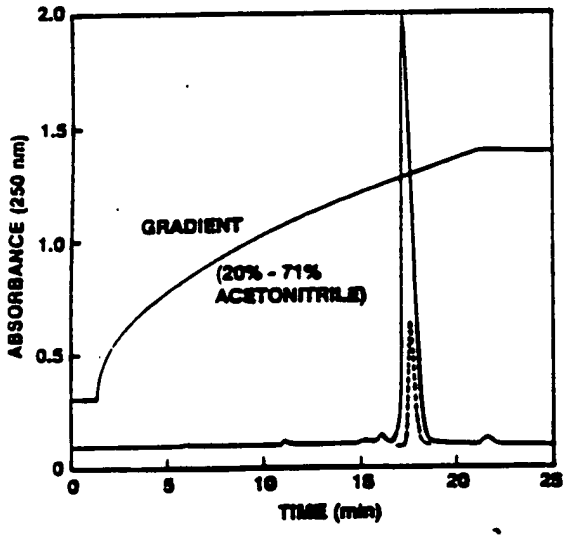


FIG. 2. Second reverse-phase preparative HPLC of enzyme inducer activity from SAGA broccoli. The active inducer bands obtained from two or three preparative silica TLC plates (see text) were combined, eluted with acetonitrile, filtered twice through 0.22-μm porosity filters, and evaporated to dryness on a vacuum centrifuge. The residue was dissolved in 0.5 ml of acetonitrile and applied to a reverse-phase preparative HPLC column (Whatman: Partisil DS-2; 50 × 1.0 cm), which was developed with a convex gradient (Waters Gradient program 5) of acetonitrile/water from 20:80% to 71:29% (vol/vol) at a flow rate of 3.0 ml/min during a 20-min period. The eluate from 17.0 to 19.0 min was collected as a pool and assayed for inducer activity; 99% of the inducer activity was recovered in this pool. The elution position of (*R,S*)-sulforaphane is shown (—).

Relation of Structure to Inducer Activity Among Sulforaphane Analogues. To define the structural features of sulforaphane (chirality, state of oxidation of sulfur of the thiomethyl group, number of methylene bridging groups) important for inducer activity, we synthesized (*R,S*)-sulforaphane and the following analogues and measured their inducer potencies: $CH_3—S—(CH_2)_n—N{=}C{=}S$ (n = 3, 4, or 5); $CH_3—SO—(CH_2)_n—N{=}C{=}S$ (n = 3 or 5); and $CH_3—SO_2—(CH_2)_n—N{=}C{=}S$ (n = 3, 4, or 5).

Induction of QR in Murine Hepatoma Cells. The chirality of the sulfoxide does not affect inducer potency, since isolated (*R*)-sulforaphane and synthetic (*R,S*)-sulforaphane gave closely similar CD values of 0.4–0.8 μM. Sulforaphane is therefore the most potent monofunctional (see below) inducer that has been identified (19, 20). Both (*R*)- and (*R,S*)-sulforaphane were relatively noncytotoxic: the concentrations required to depress cell growth to one-half were 18 μM.

Sulforaphane and the corresponding sulfone (erysolin) were equipotent as inducers of QR, whereas the corresponding sulfide (erucin) was about one-third as active (Table 1). Oxidation of the side-chain sulfide to sulfoxide or sulfone enhanced inducer potency, and compounds with 4 or 5 methylene groups in the bridge linking $CH_3S—$ and $—N{=}C{=}S$ were more potent than those with 3 methylene groups (Table 1).

Mutants of Hepa 1c1c7 cells defective in the Ah (aryl hydrocarbon) receptor or expression of cytochrome P-450IA1 can distinguish monofunctional inducers (which induce phase II enzymes selectively) from bifunctional in-

Table 1. Potency of induction of QR in Hepa 1c1c7 cells by sulforaphane and analogues

Compound	CD value, μM		
	$n = 3$	$n = 4$	$n = 5$
$CH_3—S—(CH_2)_n—N=C=S$	3.5 (Iberverin)	2.3 (Erucin)	1.7 (Berteroin)
$CH_3—S—(CH_2)_n—N=C=S$ (O)	2.4 (Iberin)	0.4–0.8 (Sulforaphane)	0.95 (Alyssin)
$CH_3—S(=O)—(CH_2)_n—N=C=S$	1.3 (Cheirolin)	0.82 (Erysolin)	0.98

Trivial names are given in parentheses. See Kjær (29).

ducers (which elevate both phase I and II enzymes) (39, 40). When sulforaphane was tested with the BP'c1 mutant (41) (defective in transport of the liganded Ah receptor to the nucleus), and the c1 mutant (42) (which synthesizes inactive cytochrome P-450IA1), induction of QR was normal (data not shown). Sulforaphane is, therefore, like benzyl isothiocyanate, a monofunctional inducer (40) and is unlikely to elevate activities of cytochromes P-450 that could activate carcinogens.

Induction of QR and Glutathione Transferase Activities in Mice. When synthetic (*R,S*)-sulforaphane, erysolin, and erucin were administered to female CD-1 mice by gavage (25), induction of QR and glutathione transferase activities was observed in the cytosols of several organs (Table 2). Sulforaphane and erucin (in daily doses of 15 μmol for 5 days) raised both enzyme activities 1.6- to 3.1-fold in liver, forestomach, glandular stomach, and mucosa of proximal small intestine, and to a lesser degree in lung. The sulfone (erysolin) was more toxic, but even 5-μmol daily doses for 5 days elevated the specific activities of these enzymes in some tissues examined. We therefore conclude that sulforaphane and its analogues not only induce QR in Hepa 1c1c7 murine hepatoma cells but also induce both QR and glutathione transferase activities in a number of murine organs.

DISCUSSION

Two considerations prompt the belief that sulforaphane is a major and probably the principal inducer of phase II enzymes present in extracts of SAGA broccoli. First, high yields of inducer activity were obtained at each step of the isolation, and even in the first HPLC (Fig. 1) more than 60% of the inducer activity was contained in a single chromatographic peak, the biological activity of which could not be subfractionated. Second, when a totally independent method of isolation of inducer activity by high-vacuum sublimation of lyophilized broccoli (5 μm Hg pressure, 60–165°C, condenser at −15°C) was used, nearly all the isolated inducer activity was found in the methanol-soluble portion of the sublimate. Moreover, on HPLC (Fig. 2) this sublimed material gave rise to only a single isothiocyanate-containing fraction, which on TLC comigrated with authentic sulforaphane and after further purification by TLC provided a high yield of sulforaphane characterized unequivocally by NMR.

The finding that the majority of the inducer activity of SAGA broccoli probably resides in a single chemical entity, an isothiocyanate, is of considerable interest. Isothiocyanates (mustard oils) and their glucosinolate precursors are widely distributed in higher plants and are especially prevalent among cruciferous vegetables (29). Sulforaphane has been identified in species of *Brassica*, *Eruca*, and *Iberis* (29, 31).

Isothiocyanates have been shown to block chemical carcinogenesis. In rats, 1-naphthyl isothiocyanate suppressed hepatoma formation by 3-methylcholanthrene, 2-acetylaminofluorene, diethylnitrosamine, *m*-toluenediamine, and azo dyes (43–46). In mice, benzyl isothiocyanate blocked the neoplastic effects of diethylnitrosamine or benzo[*a*]pyrene on lung and forestomach (47, 48), and a variety of phenylalkyl isothiocyanates reduced the pulmonary carcinogenicity of

Table 2. Induction of QR and glutathione *S*-transferase (GST) in mouse tissues by sulforaphane and analogues

Inducer	Dose, μmol per mouse per day	Enzyme	Ratio of specific activities (treated/control)				
			Liver	Forestomach	Glandular stomach	Proximal small intestine	Lung
$CH_3—S—(CH_2)_4—NCS$ Erucin	15	QR	2.19 ± 0.06	1.64 ± 0.18*	1.72 ± 0.11	3.10 ± 0.20	1.66 ± 0.13
		GST	1.86 ± 0.08	2.51 ± 0.11	2.07 ± 0.08	3.09 ± 0.21	1.41 ± 0.11*
$CH_3—S(=O)—(CH_2)_4—NCS$ Sulforaphane	15	QR	2.45 ± 0.07	1.70 ± 0.18*	2.35 ± 0.06	2.34 ± 0.19	1.37 ± 0.14*
		GST	1.86 ± 0.08	1.98 ± 0.08	2.97 ± 0.08	2.13 ± 0.20	1.17 ± 0.09†
$CH_3—S(=O)_2—(CH_2)_4—NCS$ Erysolin	5	QR	1.62 ± 0.09	1.05 ± 0.21†	1.57 ± 0.08†	1.22 ± 0.20†	1.00 ± 0.11†
		GST	1.08 ± 0.11†	1.45 ± 0.15†	1.94 ± 0.10†	0.87 ± 0.20†	1.09 ± 0.13†

The compounds were administered to 6-week-old female CD-1 mice (4 or 5 mice per group) by gavage in indicated single daily doses in 0.1 ml of Emulphor EL 620P (GAF, Linden, NJ) for 5 days. Cytosols were prepared from the tissues 24 hr after the last treatment and assayed for enzyme activities (glutathione *S*-transferase was measured with 1-chloro-2,4-dinitrobenzene). The specific activities (nmol·min^{-1}·mg^{-1} ± SEM) of organs of vehicle-treated control mice were as follows. Liver: QR, 47 ± 0.70; GST, 1014 ± 69. Forestomach: QR, 1038 ± 155; GST, 1182 ± 74. Glandular stomach: QR, 3274 ± 85; GST, 1092 ± 81. Small intestine: QR, 664 ± 119; GST, 1372 ± 266. Lung: QR, 54 ± 5.8; GST, 439 ± 34. Data are presented as mean ± SEM. All ratios were significantly different from 1.0 with $P < 0.01$, except for *, $P < 0.05$, and †, $P > 0.05$.

the t bacco-derived carcinogenic nitrosamine 4-(methylnitrosamin)-1-(3-pyridyl)-1-butanone (49, 50). The anticarcin genic effects f previously studied isothiocyanates may be related t their capacity t induce phase II enzymes, which are involved in the metabolism f carcinogens (51–57).

It will be important to establish whether the alterations of drug metabolism observed in humans and rodents after the ingestion of cruciferous vegetables (58, 59) can be ascribed to their content of sulforaphane. The finding that this isothiocyanate is a major monofunctional inducer of phase II enzymes in broccoli also provides the possibility of clarifying the relationship between enzyme induction and chemoprotection.

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General

Cancer preventive properties of varieties of *Brassica oleracea*: a review[1-3]

Christopher WW Beecher

ABSTRACT Cabbage, broccoli, Brussels sprouts, and other members of the genus *Brassica* have been widely regarded as potentially cancer preventative. This view is often based on both experimental testing of crude extracts and epidemiological data. The experimental evidence that provides support for this possibility is reviewed for the commonly consumed varieties of *Brassica oleracea*. In a majority of cases the biological activities seen in testing crude extracts may be directly related to specific chemicals that have been reported to be isolated from one of these closely related species, thus the chemical evidence further supports the data from testing extracts and epidemiology. *Am J Clin Nutr* 1994;59(suppl):1166S–70S.

KEY WORDS *Brassica*, Brassicaceae, mutagen, antimutagen, cancer, prevention, vegetables, chemoprevention

Introduction

Although most botanists would hardly agree that "A rose may be a rose by any other name" there would be substantial agreement that a cabbage and a cauliflower may be quite the same. These vegetables, and other closely related members of the Brassicaceae family, have received widespread notice recently as public figures have disavowed their consumption and scientists have upheld them as exemplary of medicinaly significant foods. Thus, in this article we review all of the experimental evidence that suggests that there may be a cancer preventive benefit from consumption of members of these closely related and commonly consumed vegetables (1, 2). Furthermore, in view of the extensive data (3, 4) that exist for these vegetables, we will restrict ourselves to those vegetables commonly classified as subvarieties of the species *Brassica oleracea* (Table 1).

From the outset it must be realized that the published experimental data come from two different types of experimental protocols. In the first type, evidence is published that concerns tests conducted on the whole food (or from crude extracts). In the second type, tests are conducted on specific chemical compounds that have been isolated from these foods. Specifically, we will cross-correlate these two bodies of data so that, whenever possible, the specific compounds that may be responsible for an observation seen in testing a crude extract are identified. It is worth noting that this information is often not available in the original article and lends credence to the initial observation.

It is our intention to provide support for observations made on crude extract and identify those areas in which the biologically active chemical species for a given observation may not yet be identified. Although various aspects of the chemistry (5), pharmacology (6, 7), biology (8–10), and general concepts of cancer chemoprevention (11–13) have been reviewed separately, we will provide an overview approach that demonstrates the overlap between these various areas. Furthermore, it is important to recognize that many clinical trials are currently underway, (14) which, in preliminary reports, lend credence to the cancer preventative approaches (15, 16).

Relevant biological activities

The etiology of cancer follows no single track but rather is the result of an accumulation of diverse events that lead to a common endpoint, namely the uncontrolled growth of a normally quiescent cell. Nevertheless, there are generally recognized to be many common stages to the development of cancer. These stages (Fig 1) include an initial insult (or mutation) to the genetic material often delivered by a mutagen or other chemical agent but may also be inherited or possibly viral in origin. A cell that has received such an insult is said to be initiated. An initiated cell will still be quiescent and not manifest its altered phenotype until it is promoted. The promotional act may similarly take multiple forms but it fundamentally involves achieving a physiological state that signals the altered DNA to be read. Where the altered message leads to an unquenchable cycle of cellular division, the cell is considered cancerous. This aberrant equilibrium, where the cell cannot reset itself, will become a tumor if it cannot regain a "normal" or self-restrained equilibrium.

Cancer chemotherapeutic agents are directed against cancerous or fully promoted cells and seek to selectively kill the cell based on some aspect of its aberrant biochemical equilibrium. As such, all current cancer treatment is based on compounds that are toxic. An ideal cancer chemotherapeutic agent would be toxic only to cancer cells but the reality is that such specificity has not yet been acheived. Although this is clearly a suitable course when the fatality of the disease is considered, the approach to cancer

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TABLE 1
The most commonly consumed members of the genus *Brassica*

Species	Variety	Common name
Brassica campestris		Field mustard
Brassica chinensis		Bok choy
Brassica juncea		Mustard greens
Brassica napus	*var napobrassica*	Rutabaga
Brassica nigra		Black mustard
Brassica oleracea	*var acephala*	Collards
Brassica oleracea	*var acephala*	Kale
Brassica oleracea	*var botrytis*	Broccoli
Brassica oleracea	*var botrytis*	Cauliflower
Brassica oleracea	*var capitata*	Cabbage
Brassica oleracea	*var gemmifera*	Brussels sprouts
Brassica oleracea	*var gongylodes*	Kohlrabi
Brassica pekinensis	*var capitata*	Cabbage (Chinese)
Brassica rapa	*var rapifera*	Turnip
Brassica rapa	*var japonica*	Red turnip

chemoprevention must be based on a very different strategy. In view of the fact that such agents must be used prophylactically, they must exhibit few, if any, side effects and must have virtually no toxicity. In addition to these stringent requirements, it needs to be recognized that any compound that is to be considered as a cancer chemopreventive agent may also exhibit a suitable spectrum of biological activity.

In cancer chemoprevention the aim is to reduce the number of initiated cells, inhibit the promotion of initiated cells, or even reverse the promotion itself. Furthermore, each of these broad categories has many strategies that may be useful to cancer prevention. First, there are strategies that aim to reduce the initiation rate. The agents here are classified as antimutagens, desmutagens, inhibitors of enzymes that activate procarcinogens, or agents that stimulate the metabolization of mutagens to less harmful metabolites. Also included here are antioxidants because a portion of the genetic damage is likely the result of free radical damage (17–21). Second, there are strategies that aim to reverse or inhibit the promotion stages. Biological activities that act at this stage may act specifically on the promoted cell to cause it to redifferentiate and hence regain control of its own division or they may act at any of the points in one of the secondary messenger (or the related oncogene) systems that are frequently implicated as destabilizing agents. In the same light, the biological consequences of low-level inflammation or constant low-level estrogenic stimulation are similarly considered destabilizing (22, 23) and hence targets for chemopreventive approaches.

As we consider such an etiology, we can associate specific bioassays that have been described in the literature in relation to one or more of these points. Thus, as a basis for this article, we have undertaken to review the reports of relevant biological activities for *Brassica oleracea* varieties. They will be organized as discussed above. With respect to initiation stages, the majority of published literature in this area may be divided crudely into two broad groupings, namely reports of an anti- (or des-) mutagenic activity and reports of stimulation of a detoxification mechanism. With respect to antipromotion activity, there is a single report that suggests that this mechanism may play a role in *Brassica's* cancer preventive potential. However, there are many reports that may not be classified as either of these stages but

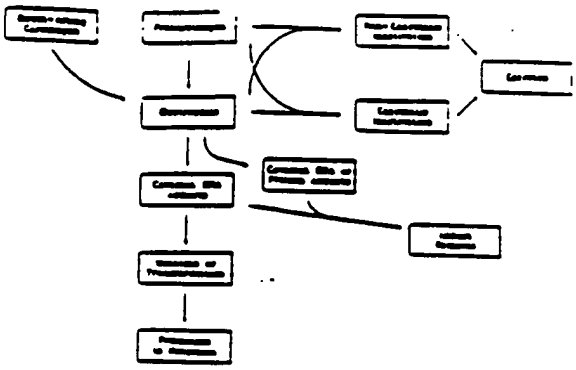

FIG 1. The etiology of cancer. The steps shown are those generally assumed in the development of cancer.

rather are of such a general nature that no specific mechanism of protection may be ascribed to them.

Antimutagenic activities

The ability of a crude extract of a *Brassica* variety to reduce the effect of a mutagen (either as a desmutagenic agent or as an antimutagenic agent) has been reported no less than eight times. In all of the cases in which a mechanism can be discerned it appears that, although the term antimutagen is used routinely, these are most likely all cases of desmutagenicity. These reports are summarized in Table 2.

The major bulk of the reports concern ability of a protein, termed the desmutagenic factor, to inhibit various mutagens in Ames-type assays. This factor, first described by Kada *et al* (24), was later characterized (25) and patented (26) by Morita et al as a heat-labile protein with a molecular weight of ≈53 kDa, which contained a prosthetic group with a heme-like chromophore. This protein was shown active against tryptophan pyrolysates (24), ethidium bromide (25), 2-aminoanthracene (25), autooxidized linolenic acid (27), and pyrolysates for other amino acids (28).

TABLE 2
Summary of antimutagenic results

Plant extracted	Mutagen	Percent reduction
		%
Cauliflower	Nitrate + methylurea	78
Cauliflower	Nitrate + aminopyrine	57
Cabbage	Nitrate + sorbic acid	Moderate (not calculable)
Cauliflower	Nitrate + sorbic acid	Moderate (not calculable)
Cabbage	Tryptophan pyrolysate	97
Broccoli	Tryptophan pyrolysate-1	97
Broccoli	Tryptophan pyrolysate-2	81
Broccoli	Ethidium bromide	92
Broccoli	2-Aminoanthracene	84
Broccoli	AF-2	0
Broccoli	Oxidized linolenic acid	82
Cabbage	Oxidized linolenic acid*	76
Red cabbage	Oxidized linolenic acid	81
Cauliflower*	Oxidized linolenic acid	76
Cabbage	Tryptophan pyrolysate-2	35

In their 1980 paper, Yamaguchi et al (27) demonstrated a striking correlation between the desmutagenic activity of the extracts and their peroxidase activity and further demonstrated that the peroxidase activity required a cofactor. This activity was later confirmed in the purified protein by Morita et al (25), who did not note the need for the cofactor. The signal characteristic to the desmutagenic factor has always been the fact that it is both heat labile and is inactivated by digestion with a proteinase. With this in mind, some workers (29) have pointed out that after heat treatment some crude extracts of *Brassica* extracts still exhibit residual activity, suggesting the presence of other antimutagenic components. Munzer (30) demonstrates that some antimutagenic activity acts by stimulating native detoxification systems in *Salmonella typhimurium* and thus some of these other agents are also desmutagenic.

The identity of the other antimutagenic agents has been the focus of other researchers. Two groups (31, 32) have reported that extracts of cauliflower and cabbage, respectively, interfere with the production of mutagens by nitrosation. There is considerable agreement that the active agents include ascorbic acid, cysteine, or other compounds acting as reducing agents. This is actually demonstrated by Osawa et al (32), who show that the ascorbic acid is responsible for the chemical reduction of the 1,2-dinitro-2-methyl pyrrole, the mutagenic nitrosation product of sorbic acid, to the nonmutagenic compound 1-nitro-2-methyl-4-amino pyrrole. Barale et al (31) show that ascorbic acid and some phenolic compounds can duplicate the activity seen in the crude extract. On the other hand, Lawson et al (33) have identified four specific compounds isolated from savoy chieftain cabbage that demonstrated antimutagenic activity against specific mutagens, *N*-methyl-*N*-nitrosourea (NMU) and 2-aminoanthracene (2-AA). These compounds, β-sitosterol, pheophytin-a, nonacosane, and nonacosanone, are notable because they are likely to be present in a majority of plants. These authors also demonstrate that commercial chlorophyll, the biological precursor to pheophytin-a, is strongly antimutagenic. These compounds were shown to present different activity profiles against the NMU and 2-AA; therefore, the authors argue that these compounds were achieving their antimutagenicity through more than one biological mechanism.

Stimulation of detoxification mechanisms

As noted briefly above, Munzer (30) noted that the antimutagenic activity of many vegetables, including cabbage, Brussels sprouts, and kohlrabi, was in stimulating the S-9 mix normally used to metabolize and sometimes activate mutagens. This observation serves to bridge the antimutagenic potential discussed above and the large body of data that makes it clear that in animals there is a strong stimulation of many of the native detoxification systems by extracts of various *Brassica* species. Although this attribute has been fairly widely discussed recently, because of the articles published by Talalay's group (34, 35), it is important to note that this area has a long and honorable background. Furthermore, although the Talalay articles do demonstrate a selectivity in the induction of phase-2 enzymes that has not previously reported, the ability of members of the Brassicaceae family to stimulate a broad spectrum of enzyme systems has been widely reported.

The earliest work on the induction of these enzyme systems was actually an attempt by Wattenberg (36) to explain variations in baseline aryl hydrocarbon hydroxylase concentrations in different rat colonies. The variation ultimately was ascribed to the presence of alfalfa as an occasional component in rat chow. This observation was followed by an examination of the ability of many foods to stimulate this enzyme. Wattenberg and his group demonstrated that many members of the Brassicaceae family were also active in this regard (37) and, furthermore, the active compounds were readily identified as indole-3-carbinol, 3,3'-diindolymethane, and indole-3-acetonitrile, which stimulated 50-fold, 20-fold, and 6-fold increases, respectively, in enzyme activities in the livers of rats that consumed augmented basal chow. In subsequent papers they demonstrated that the ability of intestinal enzymes to detoxify many xenobiotic compounds, including the indoles noted above (38), correlated to Brussels sprouts or cabbage consumption in rats (39) and in humans (40). The enzyme systems involved included many mixed-function oxidases, such as phenacetin O-dealykylase, 7-ethoxycoumarin O-dealkylase, hexobarbital hydroxylase, and benzo[a]pyrene hydroxylase. A direct correlation was later established between the induction of these activities and the concentration of these compounds by McDanell et al (41, 42). These later studies also demonstrated that the various active compounds had differing abilities to stimulate enzymes in different organs of the body. They note for instance that the ascorbic acid conjugate of indole-3-carbinol is the most active compound in stimulating the mixed-function oxidase populations of the gut whereas indole-3-carbinol, of the compounds tested, was the strongest inducer of the liver enzymes. Tanaka et al (43) demonstrated recently the ability of indole-3-carbinol to inhibit tongue carcinogenesis induced with 4-nitroquinoline-1-oxide.

Meanwhile, working in a parallel vein, Salbe and Bjeldanes (44) not only confirmed the earlier results of the Wattenberg group but also demonstrated that the enzyme glutathione-*S*-transferase was also strongly induced by Brussels sprouts. This enzyme, unlike those discussed earlier, is not a P-450 type enzyme but represents rather a phase-2 detoxification system that acts to conjugate and clear toxicants from the system. The significance of this difference cannot be understated. For most of the P-450 type enzymes, their ability to detoxify many mutagens must always be balanced by their ability to activate other mutagens (45). For glutathione-*S*-transferase, there are no such drawbacks, rather, as this group has shown (46), an increase in this enzyme alone directly resulted in an 87% reduction in the binding of aflatoxin to hepatic DNA in vivo. A wide spectrum of compounds (47, 48) including the glucosinolates, such as sinigrin and progoitrin, and their derivates, such as allyl isothiocyanate, goitrin, indole-3-carbinol, and indole-acetonitrile, induce glutathione-*S*-transferase. In other systems it is induced even more strongly by xanthoxoxin and some flavanoids (49).

Other relevant reports

There are some reports in which no mechanism can be easily ascribed to the results or that do not fit into either of the above two categories. These reports are nonetheless potentially significant with respect to the ability of brassicaceous plants to be cancer chemopreventive. The first of these concerns a study conducted by Bresnick et al (50) in which rats were fed a controlled fat diet with and without cabbage. There was found to be a statistically significant reduction in the rate of chemically induced

breast tumors in the rats with cabbage in their diet. This effect was not seen in rats on a high-fat diet. It is of interest to note that the experimental design allowed for the consumption of cabbage only after the initiation event, administration of MNU, thus indicating a potential antipromotion effect. This possibility is also suggested by a report from Koshimizu et al (51), who use the inhibition of Epstein-Barr virus induction as an indication of antipromotion activity. In their assay an extract of cauliflower is very strongly active in inhibiting the normal promotion event. In neither of these publications is it possible to ascribe a specific compound to the activity observed.

Finally, note that protease inhibitors have been associated with carcinogenesis inhibition (52, 53), so the relevance of a strong trypsin inhibitor from the seed of kale (*Brassica oleracea var sabellica*) may be relevant (54). The presence of this agent in other parts of the plant (much less in other varieties) or its ability to overcome problems of absorption and transport are totally unknown.

Summary

It may at first seem surprising that so many biological activities have been demonstrated for plants as commonly consumed as these. Yet reflection on the complex chemical nature of most plants suggests that there may be more biological potential in all of them than we would expect from something that is generally considered to be biologically neutral. Furthermore, although some of these reports have been in humans, the majority are in vivo results whose bearing on their effect on humans is very much an open question. The work of McDanell et al (41, 42) clearly demonstrates the importance of transport and the variable ability of different metabolites of even the same compound to affect different organs. A report by Birt et al (55) amplifies this by demonstrating that although the effect of cabbage is beneficial in some cases it may act to increase tumorigenicity in other model systems (or cancer types).

We have presented a case that strongly implies that the cancer preventive potential of many members of the Brassicaceae family is strong, yet it must always be stressed that to understand the relevance of these reports on the human condition, many further studies need to be done to specifically address questions of the stability, bioavailability, transport, and metabolism. The additive or even synergistic effects of these compounds are unknown. The additional effects of normal food preparation procedures present another factor that is yet largely unexplored with respect to the cancer preventive properties. In brief, there is much exciting potential in the cancer preventive properties and yet there is, as of this writing, no absolute statement that can be made concerning the ability of these foods to directly alter the course of carcinogenesis.

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